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CHEMICAL CONSTITUENTS OF THE SCENT OF MEPHITIS MEPHITIS AND MUSTELA VISON

DAVID THEODORE BERNSTEIN

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CHEMICAL CONSTITUENTS OF THE SCENT OF MEPHITIS MEPHITIS
AND MUSTELA VISON

University of New Hampshire

PH.D.

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CHEMICAL CONSTITUENTS OF THE SCENT
OF MEPHITIS MEPHITIS AND MUSTELA VISON

by

DAVID THEODORE BERNSTEIN

B. S., University of Rhode Island, 1970

M. S., University of New Hampshire, 1974

A DISSERTATION

Submitted to the University of New Hampshire

In Partial Fulfillment of

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Department of Chemistry

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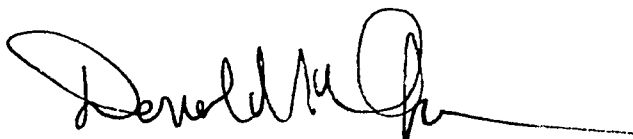
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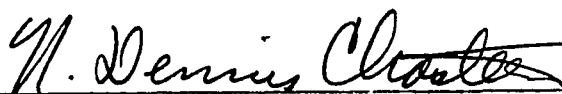
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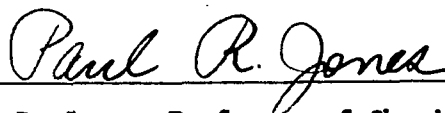
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ABSTRACT

CHEMICAL CONSTITUENTS OF THE SCENT OF MEPHITIS

MEPHITIS AND MUSTELA VISON

by

DAVID T. BERNSTEIN

University of New Hampshire, September 1979

This study continues the investigation of the chemical constituents of the anal sack secretions of the striped skunk (Mephitis mephitis) and the American mink (Mustela vison). Mass spectral analysis of the skunk anal sack volatiles showed 1-butanethiol, crotyl isopentyl sulfide, crotyl propyl sulfide, crotyl isopentyl disulfide, and dicrotyl disulfide to be present as minor components. The large number of less volatile compounds were mostly aromatic, possibly substituted quinolines. The analysis of mink anal sack secretion showed the most volatile compounds to be 2,2-dimethylthietane and diisopentyl disulfide. The less volatile compounds were found to be triglycerides.

INTRODUCTION

One of the most interesting and perhaps the most diverse group of animals in North America is the Mustelidae family, commonly called the weasel family. The animals in this family range in size from the three foot, sixty pound wolverine (Gulo gulo), to the nine inch, two ounce weasel. Most members of the family are very agile and aggressive hunters, although the wolverine relies on strength, being more aggressive than fast. The skunk is the most docile member of the Mustelidae family, taking a nonaggressive attitude and relying on a very unpleasant and irritating spray as a means of defense. The other North American members of this family include the badger, ferret, stoat, fisher, mink, otter, and martin.

The martin (Martes americana) and the fisher (Martes peunanti) are both solitary and range over Canada and the northern United States. The fisher, thirty to forty inches in length and brown to black in color, is larger and darker than the martin. When the fisher moves into new hunting territory, it will quickly eliminate any cats from neighboring farms.

The weasel (Mustela rixosa) is the smallest, but by far the most vicious member of the Mustelidae family. The short-tailed weasel or stoat (Mustela ermina) is about six inches larger than M. rixosa and is more gregarious. The white phase of the short-tailed weasel, its winter coloration, is considered valuable. Consequently, this mustalid is extensively trapped for its fur.

The clown of the family is the otter (Lutra canadensis). Others are commonly seen along rivers and streams with high banks where they make mud slides to the water. They average about four feet in length and are nomadic, feeding at one place and then moving to new sites by water or over land.

The mink (Mustela vison) ranges over most of the North American continent. The male is approximately seventeen to twenty-four inches long and the female is about half that size. Mink are nocturnal, hunting on land and in the water with equal ease. They prey on cray fish, frogs, fish, rodents, and birds. In some areas where they are not indigenous but have escaped from mink farms, they have become a nuisance by raiding poultry farms or wiping out local populations of game birds. A male mink will wander long distances, and during mating season will mate with several females as he passes through their territories. Eventually, he usually stays with one female. The females maintain small home ranges, making dens among rocks, in hollow trees or in the burrows of other animals. There are usually five or six kits in a litter born sometime in May. The family stays together until autumn when the kits have matured. The European mink (Mustela lutreola) is rarer than the American mink and its fur is inferior to that of the American mink, so American mink were imported to Europe for breeding. Some of these imported mink escaped from captivity and now outnumber the European mink in many areas.

Mink were first commercially bred in the United States in 1866¹. Since then, several colored varieties have been introduced by selective breeding. Wild mink vary in color from light brown to dark brown. Selective breeding has produced colors varying from silver

to black. Because of its commercial value, the mink has been studied more than any other member of the Mustelidae family.

The most well-known members of the weasel family in North America are the group of animals commonly referred to as skunks. The name skunk alludes to a group of animals comprised of three genera, Spilogale, Conepatus and Mephitis. Within the three genera there are thirty-three recognized varieties. The most common species of the three genera is Mephitis mephitis, the striped skunk, which ranges from southern Canada down through most of the United States to northern Mexico. The coloration and size of this mustelid vary throughout its geographical distribution. In some locations the striped skunk reaches a total length of the thirty-two inches and a weight of twelve pounds. Its coloration varies from nearly all black with a small white "V" on the back of the head to almost an entirely white back and tail. The hooded skunk (Mephitis macroura), which is the southern relative of the striped skunk, has two major color variations. One is completely black on the back with a white stripe on each side, while the other variation is almost all white on the back and tail. A broad cape of long white and black hair gives this skunk its common name².

Striped skunks feed mostly on insects, fruits, eggs and carrion; sometimes they will kill small rodents. They are mostly nocturnal, spending the major part of the day in the den. The young are generally born in May and reach maturity in approximately two and a half months. During the winter in the northern United States and southern Canada, the striped skunk spends its time in a den which is below the frost line. There are generally three to four skunks in a den although as many as ten or twelve in the same den is not uncommon.

Skunks do not hibernate, but they sometimes are inactive for days or even weeks at a time, depending on temperature, storms, and snow accumulation. If the winter is very severe, a fair number of skunks will die of starvation.

The western and southern cousin to Mephitis mephitis is the spotted skunk, Spilogale putorius. Spotted skunks are the smallest of the three genera, with the pygmy skunk being the smallest of the Spilogale genus. S. putorius reaches an average size of twenty-three inches, while the pygmy skunk averages only ten inches. Spotted skunks are considered the most primitive of the skunks because of their weasel like appearance. Of the three genera of skunks, the spotted skunk has the most complex pattern of stripes and spots. This complex coloration makes the spotted skunk almost invisible at night if it remains motionless. The contrast in coloration between the striped skunk and the spotted parallels their habits. The spotted skunk is active, aggressive, and a good climber. It is agile and hunts like a weasel, rarely being out of the cover of rocks or bushes. The striped skunk, on the other hand, is a rather docile but bold animal, often venturing from cover into open areas. Its distinctive stripes make it highly visible at night and probably serves as a warning coloration rather than camouflage. They are not as agile as spotted skunks and are comparatively poor climbers. In suburban areas the striped skunk is sometimes a pest since it may make its den under porches and other structures, dig up lawns for grubs, and get into garbage cans at night. It is not uncommon to see a skunk in the company of a cat in these areas. When threatened, skunks give a raised-tail warning and beat the ground with their front feet, but only the spotted skunk faces the threat and sprays from a hand-stand

position.

All members of the Mustelidae family have scent glands and are capable of spraying their anal sack fluid. The skunk is the only member of the weasel family in North America that uses its scent primarily for defense. Most other members use the scent for marking territory, mating, and only sometimes for defense. The striped skunk³, zorrino⁴, teledu⁵, European polecat (Mustela putorius)⁶ and the American mink⁶ are the only mustelids that have had their anal sack secretions chemically investigated.

HISTORICAL

From ancient times to the present day, man has spent a good deal of time and money pursuing ways to make his surroundings and himself more pleasant to the nose. In ancient times perfume first came into general use in ceremonial rites. In Egypt, approximately 2000 B.C., perfumes were used for personal grooming as well as for religious ceremonies and burial rites. Many of these perfumes were derived from aromatic gums taken from trees and scrubs. The gums and the finished products were important commodities in the trade between Egypt and the eastern areas.

Besides the plant-derived perfumes, animal musks were also important and in some areas were even more highly prized, for example, in Arabia the mosques Iparie at Kara Amed and Zobiade at Tauris were constructed using scented mortar⁷.

In Greek and Roman times, the use of perfumes for personal use peaked during the reign of Ceasar. In Europe, use of perfume reached its height in the court of Henry VIII.

Throughout history, there have been many plant bases for perfumes, but only three main animal sources - the civet cat, musk deer, and whales. The civet cat, which ranges throughout Africa and India, produces a vile-smelling paste in two glands located near the anal region of both the male and female. This paste is collected, and, when greatly diluted, is pleasant to the nose. The musk deer is found throughout Siberia and Tibet. The males have a pouch on their abdomen which secretes a pungent red jelly-like substance which is the musk.

The musk is obnoxious when fresh, but becomes sweet-smelling as it dries. Besides its use as a perfume, it is used as an aphrodisiac and analgesic in China. In Tibet, it is used in snuff. Probably the most interesting of the three is ambergris, the digestive by-product of the sperm whale. Ambergris has a very offensive odor when fresh, but this changes to a sweet earthy smell when it is cured. In ancient times it was worth its weight in gold. Even now, it is still valuable. A nine hundred eighteen pound chunk that was taken from one whale in 1953 brought approximately one hundred thousand dollars¹.

There has always been a quest to find the fragrance that will transcend all other earthly aromas and transport our olfactory sense to Nirvana. New fragrances from natural sources and the chemical analysis of natural mixtures has resulted in a large variety of natural and synthetic perfumes.

Although some of the worst smelling animal secretions become the most sought after perfumes when diluted and cured, a large number of scents are able to maintain their characteristic stench no matter what the treatment. Among some of the foulest smelling odors are the anal sack secretions that characterize the Mustelidae family. Of the many members of this family, the odor of the North American skunk is without a doubt the most familiar. The odor is often encountered along highways, owing to the skunks unsuccessful attempt to cross the road or the odor is worn home by the family dog. Another member of the Mustelidae family that is familiar, because of its fur rather than smell, is the mink.

Although the skunk's scent has made it the most familiar member of the Mustelidae family, many people consider mink scent to be far worse smelling than skunk scent. Both the skunk and mink have been

trapped for their fur in the past, but the use of skunk fur for coats only lasted a short time because of the characteristic skunky odor that would pervade the fur during humid weather.

Certain areas of North America were known for their populations of skunks even before the colonies were settled. The city of Chicago derives its name from an adaptation of the Fox indian phrase meaning "the place of the skunk"⁸.

Interest in the chemical nature of the anal sack secretions of the Mustelidae family began in 1862 when the first analysis of skunk scent was reported by F. Wohler, although the actual work was done by a colleague, "Dr. Swarts aus Gent". A sample of skunk scent had been sent to Wohler by friends in New York. According to Wohler, the scent had been taken from the species Mephitis mesomelas. Swarts steam-distilled the scent and obtained two volatile compounds that had a smell similar to allyl sulfur compounds. Analyzing the compounds for sulfur by the Carius method (barium sulfate), Swarts found approximately sixteen percent sulfur⁹. Wohler mentions, but does not reference, work done previously by Lassaigne who found the sulfur content to be eight percent.

Ten years later, Dr. O. Loew obtained samples of scent while on an expedition through Texas. Opposition from companions and colleagues forced Loew to abandon any further investigations of the scent¹⁰.

In 1896, two reports were published concerning the identification of the same organosulfur compound from the anal sack secretions of two different members of the Mustelidae family. Ernest Beckmann⁵ obtained anal scent sacks of the teledu (Myclaus marcheii Huet), a

Phillipine member of the Mustelidae family (R. Van Gelder, from the American Museum of Natural History, mentioned recently that the teledu, which is thought to be closely related to the badger, might in fact be a skunk)¹¹. Distillation of the scent gave Beckmann a liquid that boiled at $97^{\circ} - 105^{\circ}\text{C}$ and smelled somewhat like garlic. He dissolved the distillate in concentrated alkali and was able to regenerate the smelly oil by neutralization with acid, indicating a thiol. The distillate was then converted to the sodium mercaptide and treated with butyl bromide. The resulting compound was oxidized to the sulfone and compared with independently synthesized di-n-butyl sulfone. He concluded from mixed melting points of the sulfones, that the isolated mercaptan was n-butyl mercaptan.

Thomas Aldrich published the other report of finding butyl mercaptan in a member of the Mustelidae family¹². He obtained the scent from the species Mephitis mephitis, the striped skunk, in sufficient quantity to perform a number of tests and reactions. From the odor, behaviour in alkali, and vapor tests using lead acetate, Aldrich concluded that a major component of the scent was a mercaptan. After filtering the scent, distillation yielded fraction A, which boiled at $100^{\circ} - 130^{\circ}\text{C}$ and the residue B. Fraction A was fractionally distilled to yield two fractions and the residue. The lowest boiling fraction (α) was analyzed by several procedures. Sulfur was determined by the Carius method. Carbon and hydrogen were determined by first converting α to lead and mercury compounds. From comparison of the found values for carbon, hydrogen, lead and mercury to the calculated values for the butyl compounds, Aldrich concluded that α was in all probability a butyl mercaptan. Aldrich compared the smell of synthetic

3-methyl-1-butanethiol to that of fractions α and β , but went no further in his chemical analysis. The residue of the fractional distillation was found to contain nitrogen and sulfur, although the number of compounds and the types of functional groups present were not investigated beyond the point of determining that mercaptans were absent.

It wasn't until forty-one years later that the next report of a chemical analysis of a mustelid's anal sack secretion was published. Fester and Bertuzzi obtained the scent of a South American mustelid of the genus Conepatus suffocans, commonly known as the zorrino⁴. They steam distilled the scent, then extracted the distillate with ether. A yellow liquid with a vile odor was obtained after evaporation of the ether. The yellow liquid upon treatment with base and with nitroprusside gave indications of a mercaptan. Elemental analysis of the liquid for carbon, hydrogen and sulfur yielded the data in Table I. From the average of their data, Fester and Bertuzzi concluded that "our material is probably crotyl mercaptan, oxidized mostly to the disulfide".

Table I

	Found ^a		Calculated ^b	
			C_4H_8S	$(C_4H_7S)_2$
%C	55.86 56.00	57.44*	54.50	55.12
%H	7.47 7.63	7.76*	9.14	8.09
%S	33.78	34.80*	36.37	36.79

^aRef. (4)

*These average data were taken from Ref. (4) adjusted to total 100

^bCalculations using 1973 atomic weights

The data in Table I show discrepancies between the found and calculated values that are too large to be acceptable.

In 1945 Stevens¹³ reinvestigated the striped skunk anal sack secretions in the hope of finding a macrocyclic ketone similar to civetone. Stevens obtained a fairly large sample of scent, and stored it for several months. During the time the scent was stored, it separated into two layers with a whitish suspension at the interface. He analyzed the oily top layer for large cyclic ketones, but was unsuccessful in finding any. The scent was treated with ethanolic mercuric chloride for several days. The resulting solid was then filtered and the filtrate concentrated, added to water, extracted with petroleum ether and dried. Distillation and subsequent treatment of the first fraction with concentrated ethanolic mercuric chloride yielded a precipitate. Comparison of this precipitate to one prepared from synthetic dicrotyl sulfide lead Stevens to conclude that the first fraction was dicrotyl sulfide.

Steven's paper was the last report of work done with the anal sack secretion of the striped skunk until 1975 when Andersen and Bernstein³ reported new data that showed the major components of the anal sack secretion to be crotyl thiol, isoamyl thiol and crotyl methyl disulfide. The analysis showed n-butyl mercaptan to be absent from the secretion. Examining the data from Aldrich's elemental analysis, Table II, the calculated and found values of the mercury and lead agree more closely with those for the butyl salts than for the butenyl salts, whereas the values for carbon and hydrogen agree more closely with those for the butenyl salts. 1-Butanethiol has for years been stated as the chemical responsible for the odor of skunk scent. This is probably due to Aldrich's work, although he did not actually state that 1-butanethiol was present. Stevens stated in his paper in 1945 "it re-

mained for Aldrich to show that the principle odoriferous material is n-butyl mercaptan". Beckmann's work with the teledu was cited in 'The Merck Index', eighth edition, as the source for the statement that 1-butanethiol is in skunk scent. These cases and others have led to the misbelief that 1-butanethiol is responsible for the odor of skunk scent.

Although the mink has received considerably more scientific attention than the skunk, nothing was known about the anal sack secretions until 1975. Prior to this time, the vast amount of physiological and nutritional work reported on the mink ignored the anal sack and its contents. In 1975, Sokolow¹⁴ reported isolating by chromatographic methods several volatile aliphatic acids from the anal sack secretion. The most abundant acid that was found was butyric acid. The other acids that were isolated were acetic, propionic, isobutyric, isopentanoic, 2-methyl-butyric and a small amount of pentanoic. Sokolow also reported the amine composition of the vaginal secretions of the mink during the estrous cycle. It was found that there was a sharp increase in the amount of amine present during estrous. Of the many low molecular weight amines, triethylamine and pyridine comprised ninety percent of the amines found during estrous. A similar study of the volatile acids present in the vaginal secretions showed the presence of sixteen compounds, fourteen of which were identified¹⁵.

In 1976, H. Schildnecht¹⁶ published a communication reporting the volatile sulfur compounds in the anal sack secretions of the American mink, Mustela vison. At the time the communication was published, research on the same topic was being concluded as a portion of this thesis.

Table II

Compound		% Carbon	% Hydrogen	% Metal
(RS) ₂ Pb				
Found ^a		25.45, 25.31	3.95, 3.89	53.22, 53.02
Calcd.	(R=C ₄ H ₉)	25.00 ^a , 24.92 ^b	4.68 ^a , 4.71 ^b	53.64 ^a , 53.74 ^b
	(R=C ₄ H ₇)	25.19 ^b	3.70 ^b	54.31 ^b
(RS) ₂ Hg				
Found ^a		25.90	3.88	...
Calcd.	(R=C ₄ H ₉)	25.40 ^a , 25.36 ^b	4.76 ^a , 4.79 ^b	
	(R=C ₄ H ₇)	25.63 ^b	3.76 ^b	
RSHgCl				
Found ^a		61.62, 61.94
Calcd.	(R=C ₄ H ₉)			61.63 ^a , 61.68 ^b
	(R=C ₄ H ₇)			62.06 ^b

^aRef (12)^bCalculations using 1973 atomic weights.

It is a common misconception that mink oil, as well as skunk oil is derived from the anal sack secretions. The mink oil that is frequently seen for sale as a water proofing for leather goods is obtained from the fat of the animal, not the anal sack secretions. The same is true of skunk oil. Although skunk scent and skunk oil are not usually seen for sale, the scent has been reported to have been used for medicinal purposes. Audubon wrote:

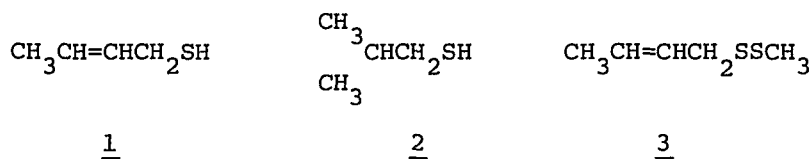
"We were once requested by a venerable clergyman who had for years been a martyr to violent paroxysms of asthma to procure for him the glands of a skunk, to be kept tightly corked in a smelling bottle, and to be inhaled when the symptoms appeared. For some time he found relief from his disturbing complaint, but he uncorked the bottle on one occasion while in the pulpit. His congregation finding the smell too powerful for their olfactories made a hasty retreat, leaving him nearly alone in church."¹⁶

RESULTS AND DISCUSSION

The Volatiles of Skunk Anal Sack Secretions

The oily or nonaqueous phase of skunk scent can be separated into two major groups of components, the volatile compounds and the non-volatile compounds. In this study any compounds that distilled below 80°C at 0.1 mm were considered nonvolatile.

Previous analysis of the major components in the anal sack secretions showed 2-butene-1-thiol (1), 3-methyl-1-butanethiol (2), and crotyl methyl disulfide (3) to be the prevalent compounds in the skunk scent obtained from a skunk breeder in Massachusetts³. The previous separation and analysis was achieved by preparative gas chromatography. The stationary phases that were used were suited to the separation of the three major components, but also resulted in the obscuring of many of the minor and trace components of the scent.



This study is concerned with the identification of the minor components of the anal sack secretion. A stationary phase that successfully resolved many of the minor components was Carbowax 20M. Of the several percentages of Carbowax 20M that were tried, a twenty percent stationary phase yielded the greatest number of minor components with the best resolution. The total oil phases of each of the five skunks used in this study were compared to determine if any differences in composition existed. The gas chromatograms of each of the

five samples were identical in the number of components and approximate relative amounts. (See Appendix GC No. S1-S5).

Although resolution was improved, a twenty percent stationary phase column could not be used in conjunction with a mass spectrometer because of excessive column bleed. Since the resolution increased as the percentage of stationary phase increased, it was thought that the hydroxyl ends of the Carbowax molecule were possibly more important than the long methylene ether chain in effecting a separation. Changing from Carbowax 20M to the lower molecular weight Carbowax 1500 increased the number of hydroxyl groups per unit of weight and also lowered the viscosity of the stationary phase at the operating temperatures. With Carbowax 1500 the resolution was increased and the stationary phase loading was decreased to five percent (see Appendix GC No. S6). Even though the Carbowax columns improved resolution of the minor components, they did not eliminate the problem of overlapping peaks. 3-Methyl-1-butanethiol (2) completely overlapped 2-butene-1-thiol (1) and crotyl propyl sulfide (1) overlapped crotyl methyl disulfide (3). A minimum of twenty-two minor trace compounds were seen as resolved peaks or definite shoulders on the chromatogram.

The same conditions that were used for the Carbowax 1500 chromatogram GC No. S6 were transferred to the gc-ms run. Once the first gc peak was detected, one mass spectrum was taken every three seconds for the duration of the chromatogram. The data were stored in a computer and were retrieved on microfilm. The data contained each mass spectral run, the ionization intensity plot for each m/e, as well as the total ionization plot for the gc-ms run.

As the amount of material in the ionization chamber increases and decreases as the peaks elute from the gas chromatograph, the overall ionization intensity increases and decreases. The maximum overall ionization intensity observed for each individual mass spectrum that was taken along the chromatographic run was plotted as ionization intensity versus spectrum number. This results in a graph that resembles the shape of the gas chromatogram. The graph shows which peak in the gas chromatogram is associated with which group of spectra. This plot of data will be referred to as the total ionization plot (see diagram Ia).

A second plot of ionization versus spectrum number involves only the intensity of a particular mass to charge peak for every spectrum along the chromatographic run. For example, the intensity of the m/e 103 peak for each spectrum is plotted versus the spectrum number (see diagram Ib).

The ionization intensity plot of m/e 103 versus spectrum number shows the maximum intensity observed along the chromatographic run to be 4505 on a scale of 1 to 9999. The entire plot shows two major peaks. The peak with the largest amplitude corresponds to an ionization intensity of 4505. The position of the peak shows that it originated from spectrum 243. The ionization intensity is overlaid on the total ionization plot (the dotted line) with the spectrum number of each plot aligned, but with different ionization intensity scales. The total ionization plot intensity scale is constant throughout the m/e intensity plots. The intensity scale for the m/e plots is adjusted so that the largest peak is full scale. A maximum observed intensity for a m/e plot of only 150 to 200 units, corresponds to the average

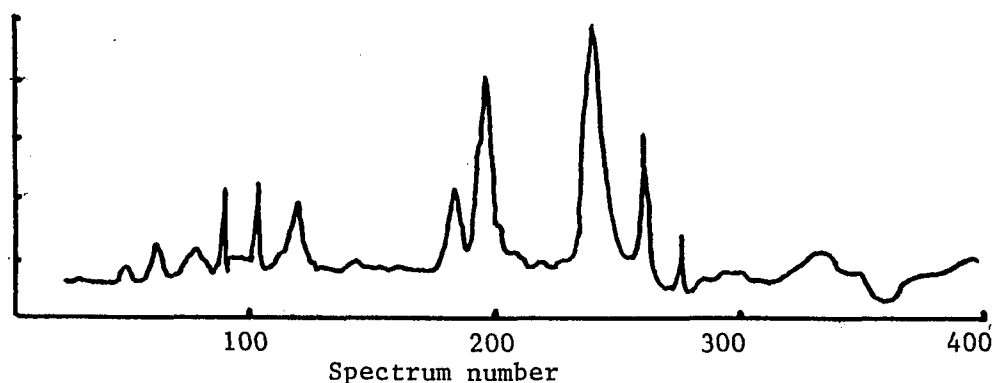
intensity of the background.

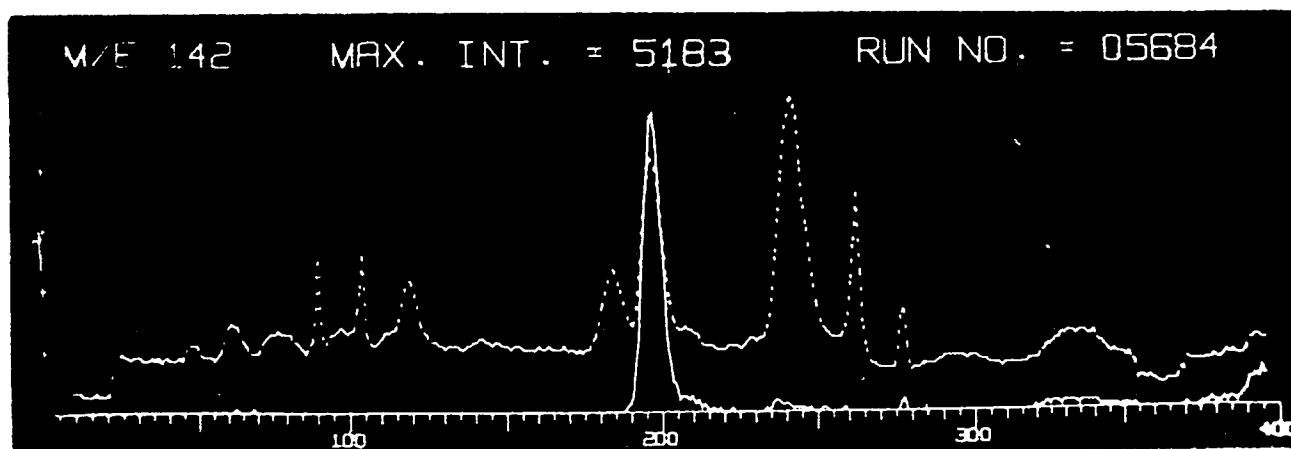
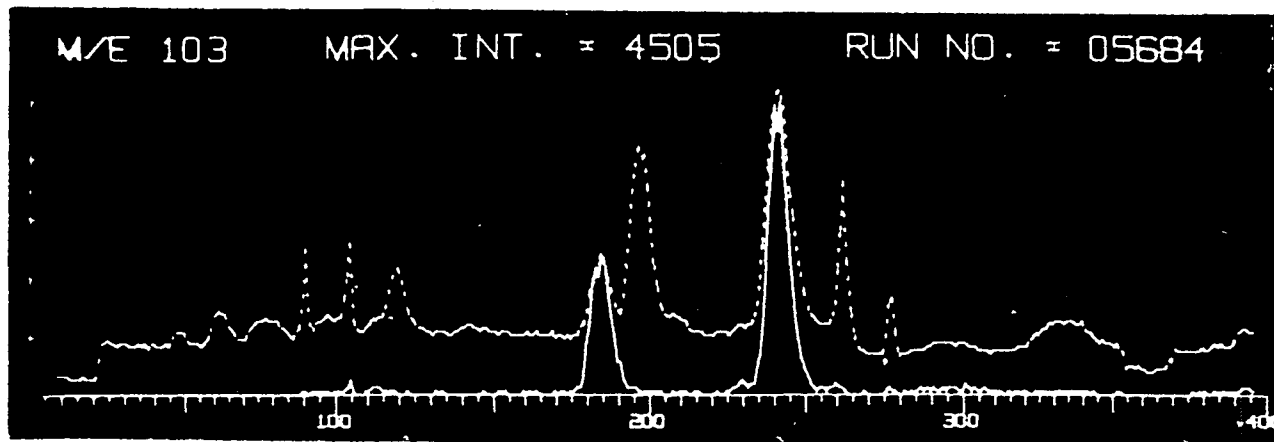
The ionization intensity plots can sometimes be used to separate data in a spectrum that are due to two overlapping compounds. Diagram Ib shows the ionization intensity plots of m/e 103 and m/e 142. If, for example, spectrum 195 is being analyzed, it is uncertain whether both the major peaks m/e 103 and m/e 142 are due to the same compound. Inspection of the intensity plots of m/e 103 and m/e 142 shows the m/e 142 plot to have a peak corresponding to spectrum 195 and a maximum in the total ionization plot which indicates a GC peak. The m/e 103 plot shows a peak with the maximum at spectrum 184 with the trailing edge at spectrum 195 that corresponds to an overlapping peak in the total ionization plot. It can be assumed that peak m/e 142 and m/e 103 in spectrum 195 are due to different compounds. If this is the case, the relative intensity of m/e 103 with respect to m/e 142 should decrease as spectrum numbers decrease.

Diagram I

Ia

Total Ionization Plot





1b

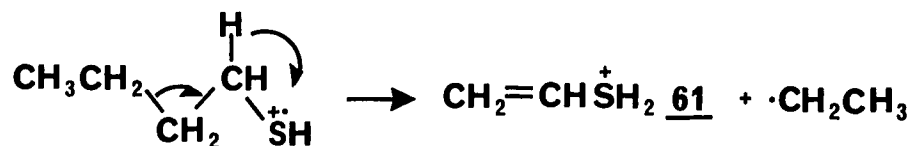
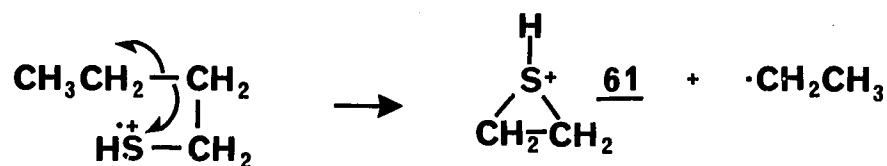
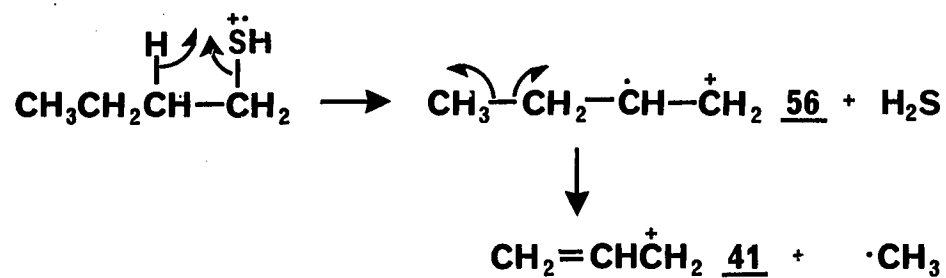
Diagram I (con't.)

Analysis of Mass Spectra

Spectrum 75, 1-Butanethiol (4). Comparison of spectrum 75 with spectra 73 - 76 shows the presence of two compounds with the compound giving m/e 90 dominating and the compound giving m/e 88 being less significant. The maximum ionization intensity for the m/e 90 peak was approximately 2300. The dominant peaks in the spectrum occur at m/e 90, 88, 61, 56, 55, 47, 43 and 41. Comparison of the ionization intensity plots of m/e 90 and m/e 88 show them to originate from two separate, but adjacent ionization maxima in the total ionization plot. The ionization intensity plots of m/e 61, 56 and 41 show them to be due primarily to the ionization maximum peak of the total ionization plot at spectrum 75. The ionization intensity plots of m/e 55 and 43 show them to originate mostly from the ionization maximum in the total ionization plot at spectrum 62. The m/e 90 molecular ion is a thiol because the base peak m/e 56 results from loss of H_2S (M-34) a process which does not occur in aliphatic sulfides^{17,18}. The molecular weight, 90, indicates a butanethiol. Comparison of the ions resulting from the ionization maximum at spectrum 75 with the reported spectrum of 1-butanethiol (see appendix Spec 75A) shows all the ions to be of the same relative intensity with the same base ion. Inspection of the reported spectra of 1-butanethiol, 2-methyl-1-propanethiol, 2-butanethiol and 2-methyl-2-propanethiol¹⁸, all shows 1-butanethiol to be the only thiol with a m/e 56 base ion. The possible fragmentation pattern of 1-butanethiol is shown in Scheme I.

SCHEME I

Fragmentation Pattern for 1-Butanethiol

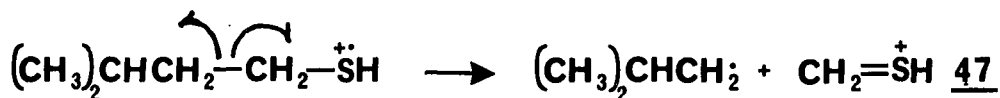
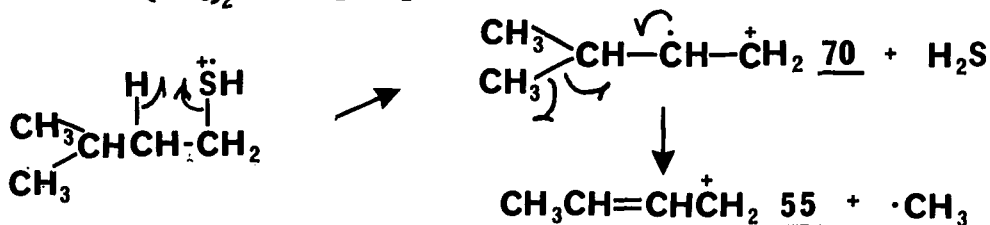
m/e 90 $\text{CH}_3\text{CH}_2\dot{\text{C}}\text{H}_2\text{CH}_2\ddot{\text{S}}\text{H}^+$ 

Spectrum 90, 3-Methyl-1-butanethiol (2). Spectrum 90 shows a molecular ion at m/e 104 of approximately 30% relative intensity. The ionization intensity plot of m/e 104, and of other major ions in the spectrum indicates negligible overlap with other compounds. The very large M-34 ion, 89% relative intensity, shows this compound to be a thiol. Comparison of spectrum 90 with the spectra of 1-pentanethiol, 2-pentanethiol, 3-pentanethiol, 3-methyl-1-butanethiol, 2-methyl-1-butanethiol, 3-methyl-2-butanethiol, 2,2-dimethyl-1-propanethiol, and 2-methyl-2-butanethiol, all of which have a molecular ion of m/e 104, shows that spectrum 90 is identical to the spectrum for 3-methyl-1-butanethiol (see Appendix Spec 90A). As with 1-butanethiol, the loss of H₂S to form the base ion is unique to 3-methyl-1-butanethiol, with a molecular ion at m/e 104. A possible fragmentation pattern is shown in Scheme II.

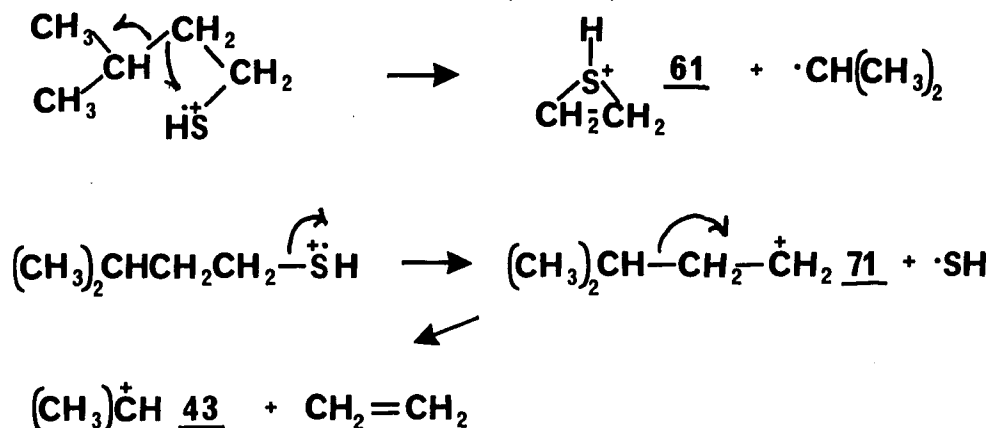
SCHEME II

Fragmentation pattern of 3-Methyl-1-butanethiol (2)

m/e 104 (CH₃)₂CHCH₂CH₂⁺SH



SCHEME II (con't.)

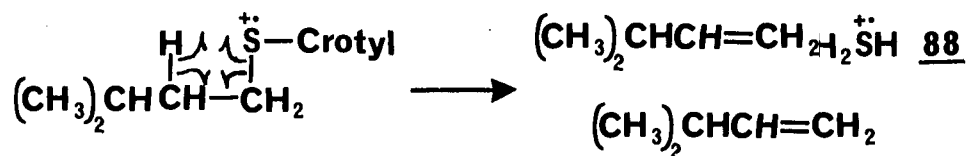
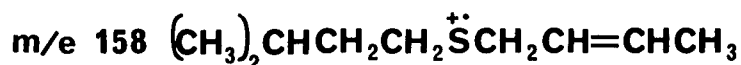


Spectrum 181, Crotyl isoamyl sulfide (5). Spectrum 181 shows a molecular ion at m/e 158. The molecular formula would be of the type $\text{C}_9\text{H}_{18}\text{X}$, where X is S or O_2 . If X is oxygen, the spectrum should show ions that are characteristic of the functional group. Primary alcohols show strong ions due to $\text{CH}_2=\text{OH}^+$ at m/e 31. A strong ion due to $\text{R}_2\text{C}=\text{OH}^+$ or $\text{R}_2\text{C}=\text{OH}^+$ (m/e 45, 59, 73, 87, ... $87+n \cdot 14$) results from fragmentation of secondary and tertiary alcohols, and also occurs for ethers. Ketones and aldehydes show strong ions at m/e 43, 57, 71, ... $(43+n \cdot 14)$ due to $\text{R}-\text{C}=\text{O}^+$. The McLafferty rearrangement will give ions at 44, 58, 72, ... $(44+n \cdot 14)$. For esters the $\text{R}-\text{C}=\text{O}^+$ fragment is an intense ion that occurs at $M-31$, $M-45$, ... $(M-31-n \cdot 14)$. Also, the McLafferty rearrangement will give a relatively strong peak at m/e 74, 88, 102, ... $(74-n \cdot 14)$. The molecular ion for esters is relatively weak in the range m/e 130 to m/e 200^{17.19}.

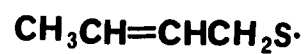
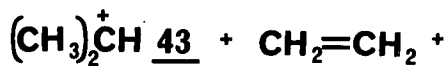
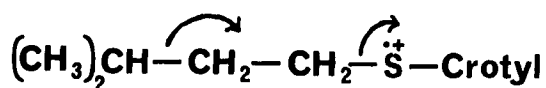
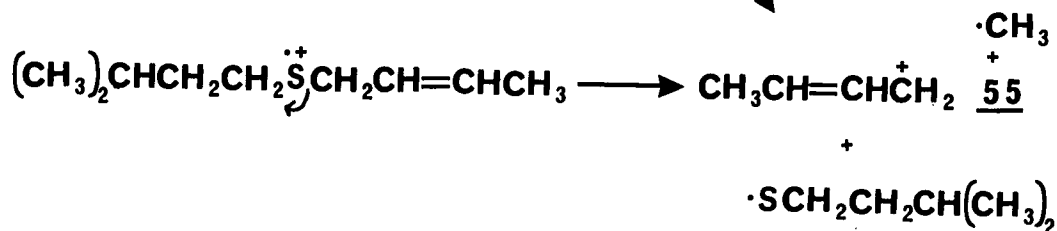
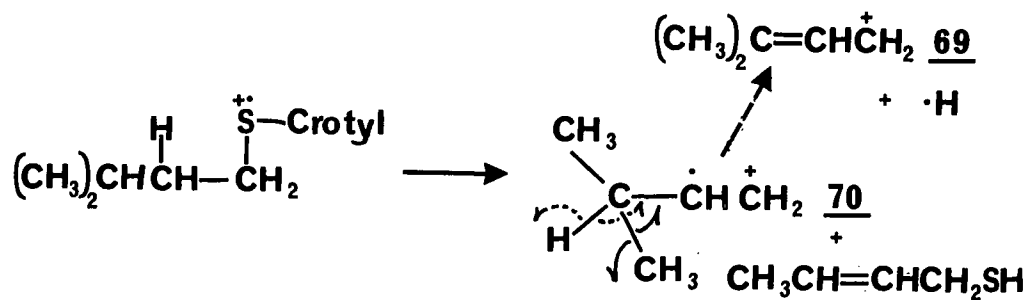
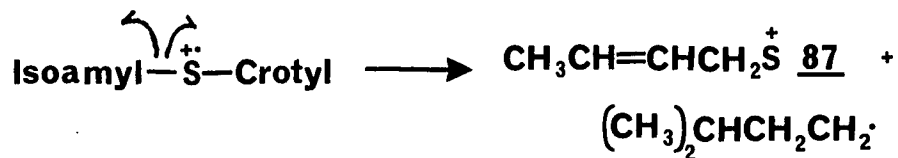
In spectrum 181 the relatively strong molecular ion and the major ions indicate the presence of sulfur rather than oxygen. Ions that characterize the oxygen functional groups are absent. The absence of an M-34 ion indicates the presence of a sulfide rather than a thiol. The molecular formula shows the presence of a ring or a double bond. Sulfides characteristically show major ions from cleavage of the sulfur carbon bond, which in many cases is accompanied by hydrogen rearrangement^{17,19}. The spectrum shows major ions at m/e 103, 88, 87, 70, 69, 61, 43 and the base ion at m/e 55. The ion at m/e 103 is due to the loss of 55, which corresponds to C_nH_{2n-1} where n is 4. This is equal to the loss of an alkenyl radical. For m/e 88 and m/e 87 there is a loss of C_5H_{10} and C_5H_{11} . The three ions m/e 103, 88, and 87 represent cleavage at the sulfur-carbon bonds with retention of the charge on the fragment containing the sulfur. The sulfur-carbon bond cleavage with retention of the charge on the alkyl fragment with a one hydrogen rearrangement results in the ions of m/e 70, 69 and the base ion m/e 55. The possible fragmentation pattern is outlined in Scheme III.

SCHEME III

Fragmentation Pattern of Crotyl Isoamyl Sulfide



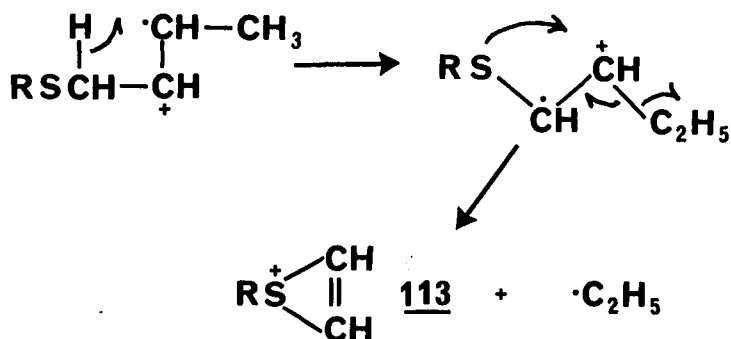
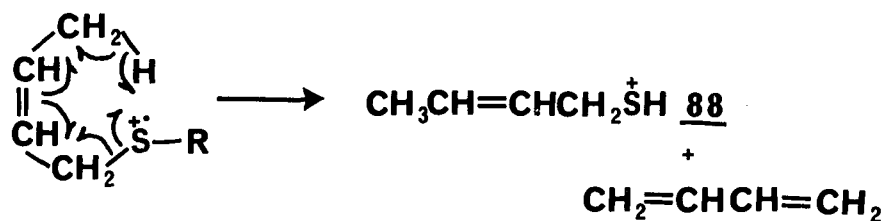
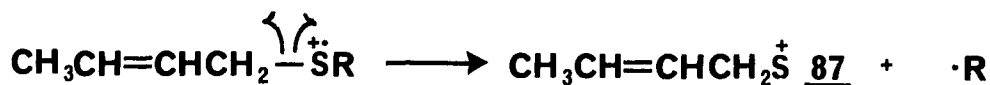
SCHEME III (con't.)



Spectrum 192, Dicrotyl Sulfide (6). Spectrum 192 shows a molecular ion at m/e 142. The molecular formula is of the type $C_nH_{2n-2}X$. The large $M+2$ ion indicates the presence of sulfur as well as the lack of ions characteristic of oxygen functional groups. The molecular formula would then be $C_8H_{14}S$, which shows the presence of two double bonds, one double bond and a ring, or two rings. The $M-34$ ion is very small, too small to be considered as evidence for the presence of a thiol. The base ion m/e 55, which in previous spectra has originated from either an isopentyl moiety or a crotyl moiety, most likely originates from an S-crotyl group because of the lack of a strong m/e 70 ion which appears to be the intermediate ion in the formation of m/e 55 from an isopentyl moiety. The m/e 88 and m/e 87 peaks together are a further indication of the presence of an S-crotyl moiety. If S-crotyl is subtracted from the molecular formula, C_4H_7 is left, this leads to the conclusion that the molecule is dicrotyl sulfide. The ion at m/e 113 seems to be an anomaly until one considers an initial loss of an electron from the pi-bond rather than the sulfur. The first step in this fragmentation is a typical four membered transition state for a hydrogen transfer due to radical site initiation^{17,19}. The second step is a charge site initiated rearrangement where the charge is transferred to the sulfur and an alkyl radical is eliminated resulting in m/e 113. The relatively small ion at m/e 88 can be explained by hydrogen transfer preference. Djerassi has shown that hydrogen rearrangement in sulfides is preferred from a secondary hydrogen that can participate in a four-, five-, or six-membered transition state, all of which are equally favored²⁰. The small ring transition state (three membered) is feasible but not preferred. In

this case the symmetrical sulfide has no secondary hydrogen available for a four-, five-, or six-membered transition state. The primary hydrogen of the methyl group can transfer through the six-membered transition state outlined in Scheme IV. The possible fragmentation pattern is outlined in Scheme IV.

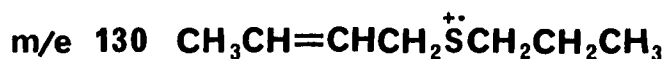
SCHEME IV
Fragmentation Pattern of Dicrotyl Sulfide



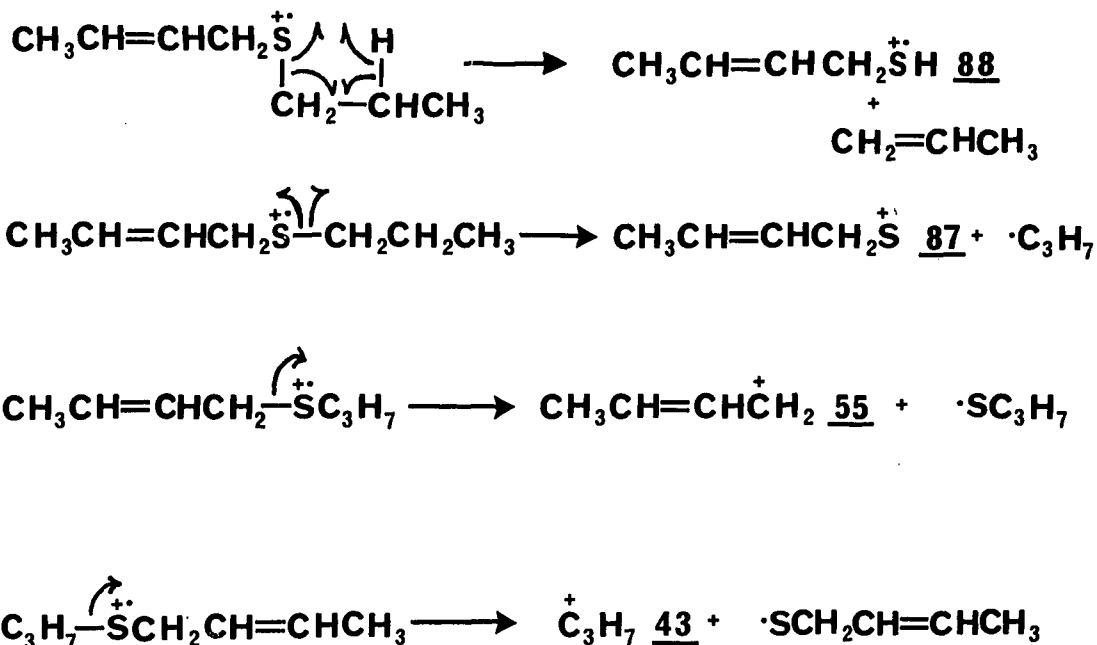
Spectrum 260, Crotyl Propyl Sulfide (7). Spectrum 260 shows a strong molecular ion at m/e 130. The molecular formula is of the type $C_n H_{2n} X$. Absence of the typical fragmentation patterns for oxygen containing compounds, plus the strong molecular ion indicate the presence of sulfur. The molecular formula shows the presence of a ring or a double bond. The major ions in the spectrum are m/e 130, 88, 87, 55 and 43. Lack of an ion at m/e 96 (M-34) indicates the compound is a sulfide. The m/e 88, 87 pair show the crotyl moiety to be present. The major ions in a spectrum of a sulfide are generally due to the sulfur-carbon bond fragmentation products. Therefore, subtracting the crotyl moiety from the molecular formula should give the second R group of the sulfide. In this case it is C_3H_7 , which corresponds to a propyl group. Inspection of the data on propyl sulfides in Levy and Stahl's paper¹⁸ shows that the two secondary hydrogens on the beta carbon of the n-propyl group versus the six primary hydrogens on the beta carbons of the isopropyl group, lead to a marked increase in the relative intensity of the M-42 ion. By analogy, the large relative intensity of the M-42 ion (m/e 88), and the large maxima in the ionization intensity plot of m/e 43, indicate the presence of an n-propyl group. A probable fragmentation pattern is outlined in Scheme V.

SCHEME V

Fragmentation Pattern of Crotyl n-Propyl Sulfide



SCHEME V (con't.)



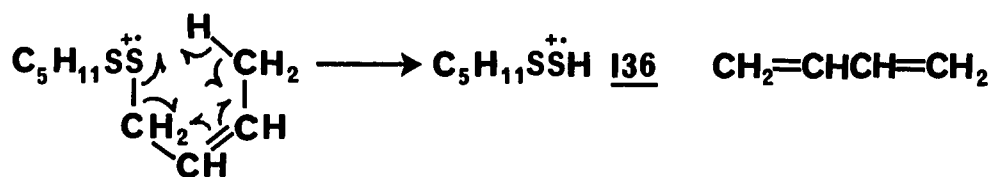
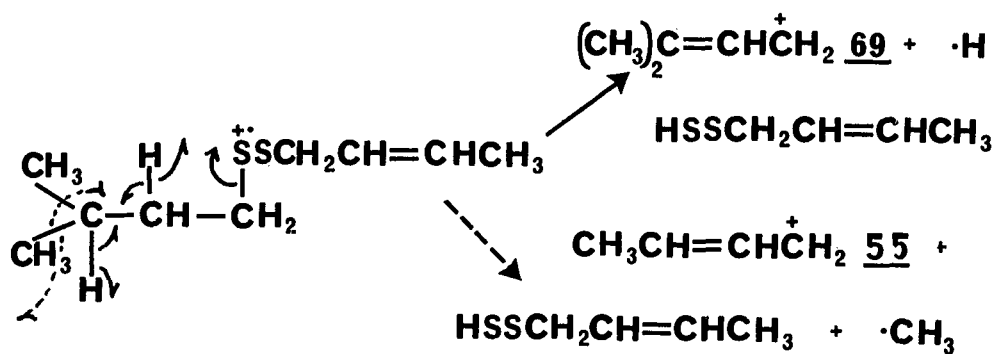
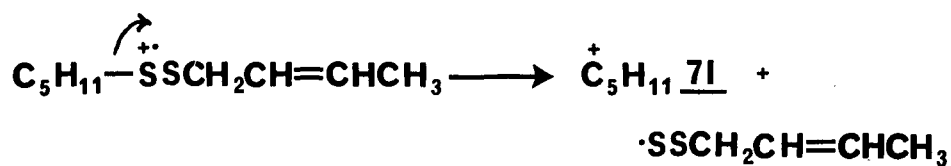
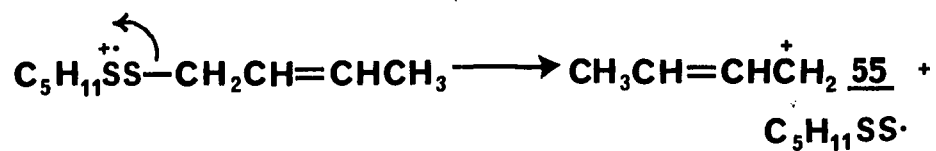
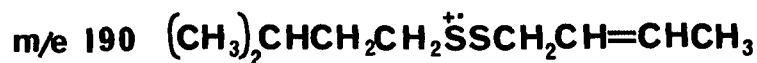
Spectrum 294, Crotyl Isoamyl Disulfide (8). Spectrum 294 shows a molecular ion at m/e 190. The molecular formula is of the type $\text{C}_n\text{H}_{2n}\text{X}$, where n is 9 and X is S_2 . The molecular formula shows there is a ring or a double bond. Assuming the two sulfurs to represent a disulfide, the dominant ions in the spectrum should arise from cleavage of the carbon-sulfur bonds, as in sulfides. Unlike sulfides, the carbon-sulfur bond cleavage in disulfides results with retention of charge mostly with the alkyl group rather than the sulfur containing fragment. The ion at m/e 136 represents a saturated fragment. Subtracting two sulfurs from this fragment yields C_5H_{11} . The remainder of the molecule must be a butenyl moiety. The strong ion at m/e 55 and the absence of the m/e 70 ion shows that the m/e 55 ion originates

mostly from the butenyl moiety and not from the pentyl moiety as was seen in other spectra. This agrees with the dominant fragmentation pathway for disulfides which occurs with retention of the charge on the alkyl fragment. Ions at m/e 71 and m/e 69 result from the alkyl fragment retaining charge, although m/e 69 also involves a radical site initiated rearrangement that could account for a portion of the m/e 55 ion. Possible fragmentation patterns are outlined in Scheme VI.

Spectrum 348, Dicrotyl Disulfide (9). Spectrum 348 shows a molecular ion at m/e 174. Considering only the molecular weight there are two formulas that are the most logical choices; i.e., $C_8H_{14}S_2$ (dicrotyl disulfide) or $C_{10}H_{22}S$ (diisopentyl sulfide). Including the molecular ion, there are only three major ions in the spectrum, m/e 174, 120 and 55. The base ion is m/e 55. Comparison of this spectrum with the spectra of diisopentyl sulfide and di-n-pentyl sulfide (appendix) show them to be completely different from one another. The base ions for the latter two sulfides are at m/e 70. They also have major ions at m/e 61 and m/e 55. There is a complete absence of any ions at m/e 120. The ion at m/e 120 in spectrum 348 originates from a radical site initiated rearrangement that involves the transfer of a hydrogen via a six membered transition state. Sulfur-carbon bond cleavage with retention of the charge on the sulfur-free alkenyl fragment results in the base ion. Possible fragmentation patterns are outlined in Scheme VII.

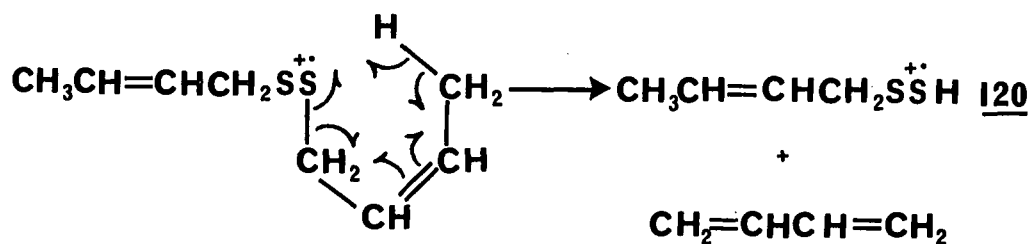
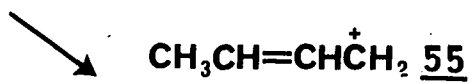
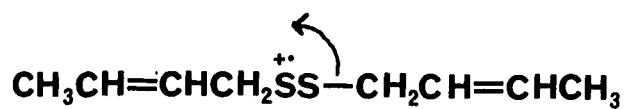
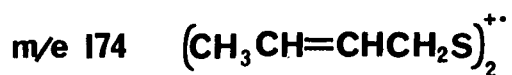
SCHEME VI

Fragmentation Pattern of Crotyl Isoamyl Disulfide



SCHEME VII

Fragmentation Pattern of Dicrotyl Disulfide



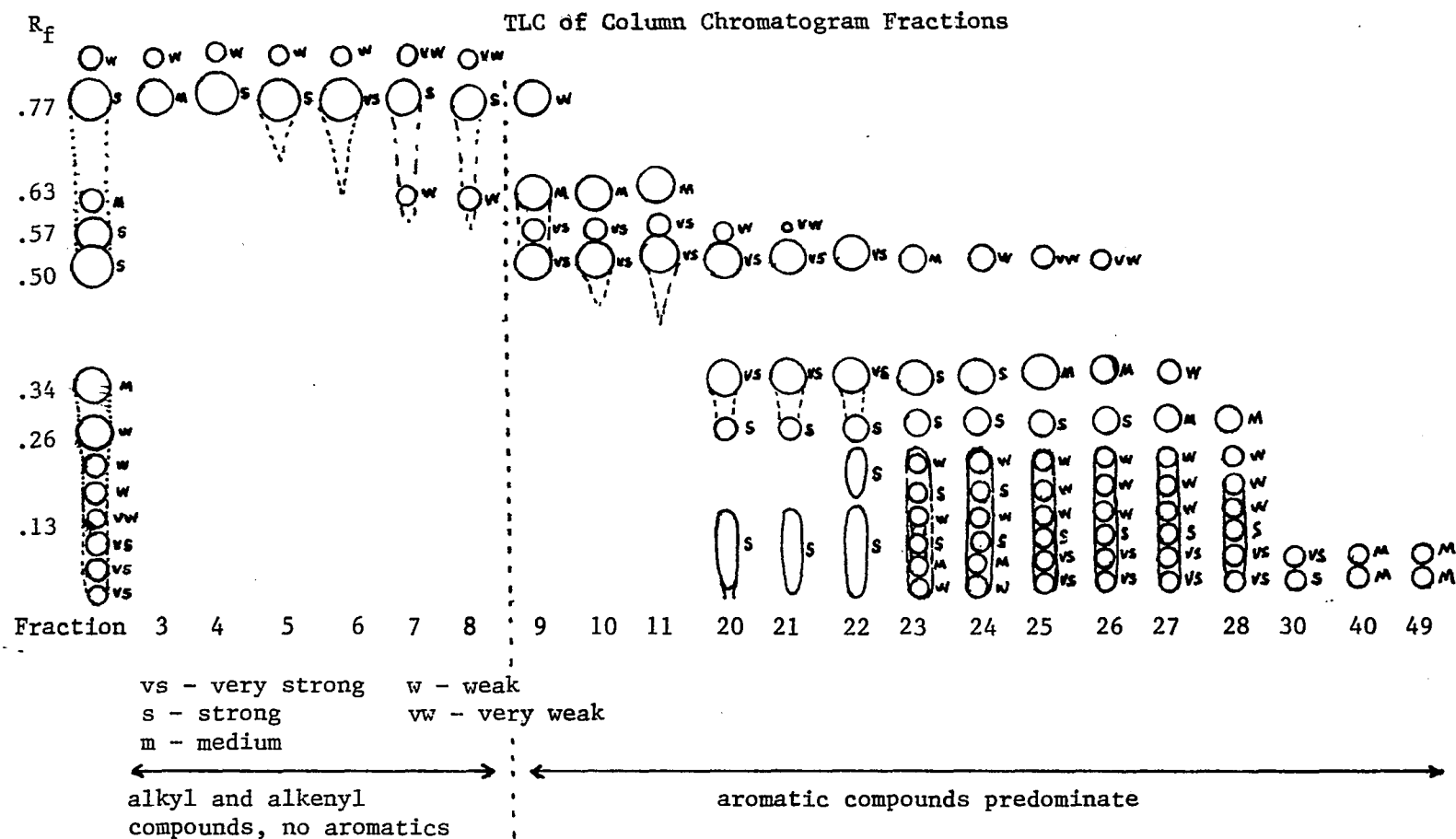
Nonvolatile Components of Skunk Anal Sack Secretion

The nonvolatile portion of skunk scent is a dark amber liquid that is made up of a number of nitrogen containing aromatic compounds. A trap to trap distillation and an nmr of the total oil show the nonvolatiles to account for approximately one third of the total oily phase. Exhaustive analysis of the nonvolatiles by thin layer chromatography showed the mixture to contain a minimum of twelve compounds. Several attempts at column chromatography using gradient elution and progressively longer columns gave unsatisfactory resolution of the components, even with a three foot column and collection of three hundred 3 ml fractions. Prechromatographing those fractions under different conditions did not improve the resolution or lessen the tailing. Part of the problem seemed to be that some of the compounds were unstable and degraded readily on silica gel.

A typical column chromatogram of the anal sack secretions, although unsatisfactory with regard to the resolution of the compounds, does show some interesting features when the thin layer chromatograms as well as the nmr spectra of each fraction are compared. The tlc obtained on fractions from a column chromatogram eluted with chloroform are shown in Diagram II. Nmr (see appendix, nmr spectra 1 and 2) showed the first three tlc spots resulted from alkyl and alkenyl but not aryl compounds. Aromatic compounds first appeared in fraction 9 (nmr spectrum 3) and dominate the remaining forty one fractions. In fraction 9, two of the spots (R_f 0.77 and 0.63) responsible for the alkyl peaks in the nmr of earlier fractions remain, but two new spots at R_f 0.57 and 0.50 have appeared. One or both of these spots could be responsible for the aromatic peaks in the nmr of the fraction. In

fraction 11 the last spot responsible for just the high field peaks in the nmr's of earlier fractions (R_f 0.63) is still present, along with the components at R_f 0.57 and 0.50 (nmr spectra 4-7). By fraction 20 all of the spots responsible for the earlier fractions high field peaks in the nmr are absent, yet significant peaks in the high field portion of the nmr spectrum (8) persist. In fraction 20 there are additional spots at R_f 0.34, 0.26 and a rather long diffuse spot from the origin to R_f 0.13. In fraction 22 (nmr spectrum 9) the spot that appeared in fraction 9 at R_f 0.57 is absent, but a new diffuse spot has appeared at R_f 0.21 to 0.15. The nmr of this fraction shows a significant decrease of most of the high field signals, but it also shows the appearance of two singlets at δ 2.4 and δ 4.47. During the transition from fraction 9 to fraction 22 the multiplicity of the aromatic signal changed significantly. It appears that some alkyl and/or alkenyl moiety is bonded to the aromatic system. Also, the appearance of a diffuse spot at R_f 0.21-0.15 in fractions 22-28 (nmr spectra 9-12) shows that one or more of the compounds between R_f 0.57 and R_f 0.26 are unstable. These unstable compounds are degrading on the column and are responsible for the diffuse spot at R_f 0.21-0.15. Fraction 27 (nmr spectrum 12) gave two new singlets in the nmr at δ 2.77 and δ 4.07, and the spot at R_f 0.50 disappeared. Although two new signals appeared in the nmr, the only visible change in the tlc, besides the disappearance of R_f 0.50, was the apparent darkening of the spots at R_f 0.03 and R_f 0.09. In fraction 30 (nmr spectrum 13) all but two spots at R_f 0.09 and R_f 0.03 had disappeared. The aromatic signal multiplicity had changed, and except for the four distinct singlets at δ 2.40, δ 2.77, δ 4.47 and δ 4.47, all of the remaining weaker signals in the high field

DIAGRAM II



Non-volatile components of skunk anal sack secretions. (eluent - chloroform)

portion of the spectrum had disappeared (see Fig. 1).

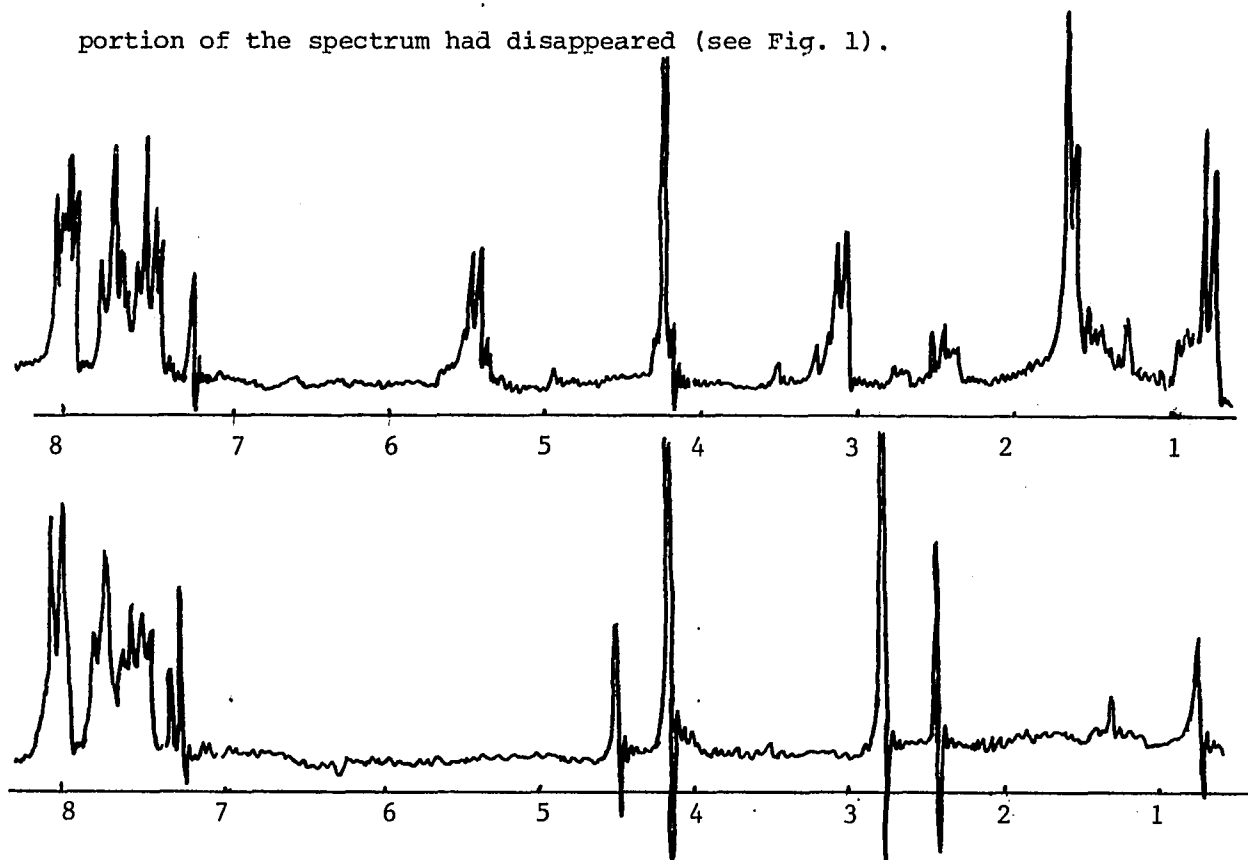


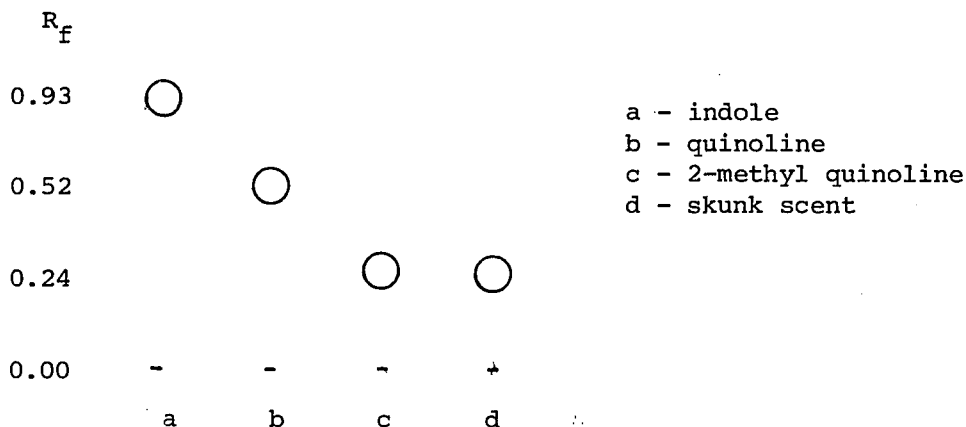
Figure 1. NMR spectra of fractions 12 and 30 from the column chromatogram of the non-volatile portion of skunk scent.

The compounds at R_f 0.09 and R_f 0.03 exhibited an nmr pattern in the aromatic region that is typical for fused ring aromatic systems²¹.

Elemental analysis of fraction 24 showed the presence of nitrogen (5.09%) carbon (63.95%), and hydrogen (6.31%). The remaining 24.65% is probably sulfur. It has been reported that indole was found in the anal sack secretions of the European polecat⁶. Comparative tlc of indole, quinoline, 2-methylquinoline and the nonvolatile portion of skunk scent, using a solvent system typical for the separation of these compounds²², showed that the skunk scent gave spots with an R_f similar to the quinolines (see Diagram III). Several of the fractions (from the above column chromatogram) were extracted with ten percent KOH. Then the KOH solution was neutralized and extracted with methylene

chloride. The organic solution was concentrated and the small amount of residue remaining was taken up in CDCl_3 . Nmr showed that no aromatics and thus no indoles unsubstituted in the 1-position were present.

DIAGRAM III



Eluent-ethyl acetate 80%, methanol 10%, formic acid 10%
Silica gel treated with sodium acetate

A series of thin layer chromatograms eluted with 3V:1V chloroform: ether was successful in resolving two of the stronger spots found at R_f .26 and .34 (Diagram II). These two spots corresponded to spots R_f 0.54 and R_f 0.38 from the preparative thin layer chromatogram. The spots at R_f 0.54 and R_f 0.38, were analyzed by nmr and uv spectroscopy. The uv λ max for compounds with R_f 0.54 and R_f 0.38, plus the λ max of some quinolines and other fused ring compounds, are listed in Table III (see appendix for actual spectra: uv spectra 1-13).

The uv spectra of the two compounds isolated from the non-volatile portion of skunk scent agree more closely with the spectra for the quinolines than with the spectra for the other compounds. Dialkylquinolines should show a bathochromic shift of about 4 nm compared to monoalkylquinolines¹⁷. This brings the numerical data for monoalkyl-

quinoline into good agreement with the data for the two unknowns.

TABLE III

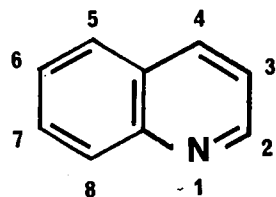
Compound	Max (nm)	Fine Structure max (nm)
R _f 0.54	230	270, 292, 298, 305, 311, 319
R _f 0.38	224	270, 290, 296, 303, 309, 317
	228, 232	
^a Indole	220	262, 267, 276, 280, 288
^a Isoquinoline	118	265, 292, 299, 304, 311, 317
^a Quinoline	226, 231	266, 278, 288, 294, 300, 306, 314
^a 2-Methyl quinoline	227, 232	270, 278, 290, 295, 303, 309, 316
^a 4-Methyl quinoline	222	273, 302, 315
^a 6-Methyl quinoline	224, 232	270, 293, 298, 304, 310, 318
^a 7-Methyl quinoline	228, 233	277, 286, 292, 298, 305, 310, 318
^a 1,7-Dimethyl naphthalene	228	280, 293, 308, 315, 322
^a 2,6-Dimethyl naphthalene	226	274, 285, 297, 303, 310, 317, 325
^a Ref. 23		

The overall appearance and the relative intensities in the spectrum in general should not change significantly from the monosubstituted quinolines to the disubstituted quinolines. The only changes should be in the fine structure, and these should be similar to those seen going from mono- to disubstituted-naphthalenes. From the uv data and the chemical data the two unknowns appear to be substituted quinolines, possibly disubstituted.

The nmr spectrum of compound R_f 0.54 shows an unresolved multiplet at 0.95, a broad singlet at δ1.30, a singlet at δ2.74, overlapping signals at δ7.4 - 8.1 and what appears to be a doublet at δ8.24 with a

coupling constant of 8.3 ± 0.5 Hz, (see appendix nmr spectrum 14). The compound with R_f 0.38 shows an unresolved signal at $\delta 0.95$, a singlet at $\delta 1.3$, a singlet at $\delta 4.2$, overlapping signals at $\delta 7.4 - 8.1$ and a doublet at $\delta 8.22$ with coupling constant of 8.3 ± 0.5 Hz (see appendix nmr spectrum 15). The apparent singlets at $\delta 1.30$ in spectra 14 and 15 cannot be due to a group directly bonded to the ring because the signal is too far upfield. The first thing that is apparent in these spectra is the absence of the 2-position proton signal at approximately 8.8 for quinoline. In the spectra of quinoline (nmr spectrum 16) and 2-methylquinoline (nmr spectrum 17) the proton signals from the 4 and 8 hydrogens are 0.3 ppm downfield from the rest of the aromatic signals for the quinoline ring. For these two compounds the $J_{3,4}$ is 8.5 ± 0.5 Hz and $J_{7,8}$ is 8.3 ± 0.5 Hz with a 5 Hz separation between the two signals. This shows up as two overlapped doublets that appear as a quartet in quinoline and 2-methylquinoline. The spectra of the compounds with R_f 0.38 and R_f 0.54 show a doublet in this region, with separations or couplings of 8.3 ± 0.5 Hz. The spectra of 2,6-dimethylquinoline (nmr spectrum 18), 6-methoxyquinoline (nmr spectrum 19) and 6-methoxy-2-methylquinoline (nmr spectrum 20) all show the 4 and 8 protons as a doublet rather than a quartet²¹. For the spectrum of isoquinoline, a large multiplet appears at $\delta 7.5 - 8.1$. H-1 appears as a singlet at $\delta 9.34$, and H-3 appears as a doublet at $\delta 8.60$. $J_{3,4}$ is 5.3 ± 0.5 Hz³³. The spectra of 1-chloro-3-methylisoquinoline and 3-methyl-1-isoquinolinol show what appear to be doublets with fine splitting at $\delta 8.28$ and $\delta 8.19$ respectively. Both of these doublets have J values of 6.0 ± 0.5 Hz²¹. Comparing the J values of the down field doublet of the substituted and unsubstituted quinolines and isoquinolines indicates that the structures

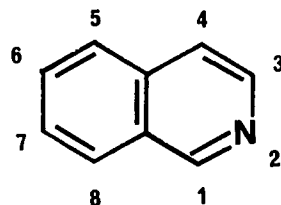
of the unknowns are probably quinolines rather than isoquinolines.



Quinoline

$$J_{3,4} \quad 8.5 \pm 0.5 \text{ Hz}$$

$$J_{7,8} \quad 8.3 \pm 0.5 \text{ Hz}$$



Isoquinoline

$$J_{3,4} \quad 5.3 \pm 0.5 \text{ Hz}$$

Volatiles from Mink Anal Sack Secretion

The volatiles were separated from the nonvolatiles by trap to trap distillation (see nmr spectrum 21 of the total volatile portion).

After the distillation was complete, the small amount of distillate, which amounted to approximately 5% of the total oil, was gas chromatographed to determine the number of components. Of the several stationary phases that were tried, only Apiezon-L proved to be satisfactory. Two compounds were found in the volatile portion of the scent. They were isolated by preparative gas chromatography using 20% Apiezon-L and temperature programming. Similar conditions were used by Schildknecht in his study of mink scent⁶.

The more volatile of the two compounds gave signals in the nmr at δ 1.65 (s), δ 2.25 (t) and δ 3.4 (t) that integrated in the ratio 3:1:1 (nmr spectrum 22). Comparison of this spectrum with the spectrum of 2,2-dimethylthietane (nmr spectrum 23) show the two to be identical.

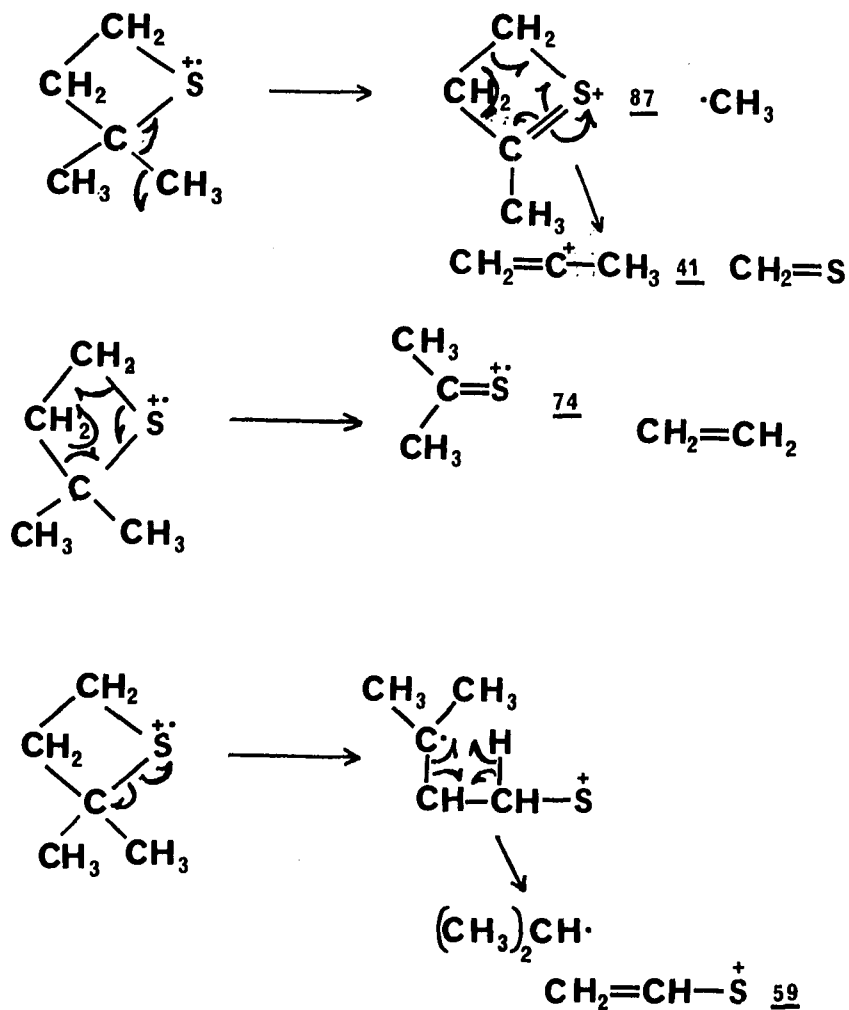
The less volatile of the two compounds gave a doublet at δ 0.94 (6H), a multiplet at δ 1.62 (3H), and a triplet at δ 2.75 (2H) (nmr spectrum 24). Comparison of this spectrum with the spectrum of diisopentyl disulfide (nmr spectrum 25) shows the two to be identical. The singlet at 2.61 is due to dimethyl sulfoxide which overlaps the upfield peak of the methylene triplet α to the sulfur. Comparative gas chromatography of known compounds with the oil phase of mink scent also showed the two volatile compounds to be 2,2-dimethylthietane and diisopentyl disulfide. In Schildknecht's communication concerning the analysis of mink anal sack secretions, he reports finding 2,2-dimethylthietane and diisopentyl disulfide, but he also reports finding 3,3-dimethyl-1, 2-dithiolane⁶. Repeated gas chromatographic analysis of the total oil phase used in this study failed to show the presence of any compounds other than 2,2-dimethylthietane and diisopentyl disulfide.

Mass Spectral Analysis of 2,2-Dimethylthietane

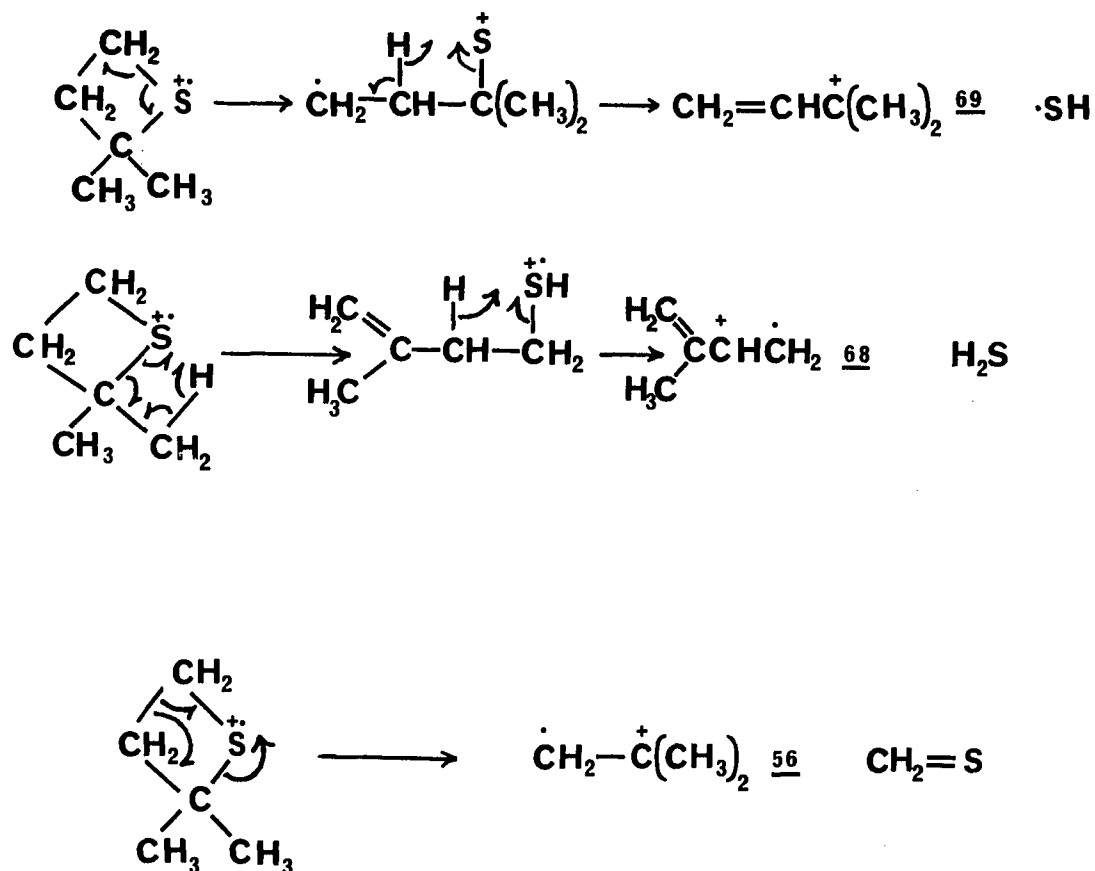
The molecular ion for this thietane is one of the dominant ions in the spectrum (mass spectrum M-1) with a relative intensity of sixty-nine percent. The other major ions in the spectrum occur at m/e 87, 74, 69, 68, 59, 56 (base ion), and 41. The base ion results from the loss of $\text{CH}_2=\text{S}$ from the parent ion. The ion at m/e 41 which has a relative intensity of ninety-nine percent, also results from the loss of $\text{CH}_2=\text{S}$, but through the intermediate ion at m/e 87. One of the first ions that stands out is the ion at m/e 68 (M-34). It is generally accepted that the presence of an M-34 ion in a spectrum indicates a thiol^{17,18}. Levy and Stahl have compiled data on 29 thiols and 31 sulfides¹⁸. For all of the sulfides listed, not one has an M-34 ion. The thiols that were listed all had an M-34 ion of relative intensities varying from a few percent to one hundred percent. This data indicates that 2,2-dimethylthietane does not fall into the sulfide category. However, if the data for cyclic sulfides in Table IV is compared to the data of Levy and Stahl, it becomes evident that the M-34 rule does not hold for cyclic sulfides. The relative intensity of the M-34 ion of these cyclic sulfides varies from 1.04 percent for thietane to seventy percent for thiacyclohexane. This shows that the M-34 ion is present in the spectra of cyclic sulfides to the same extent as in the spectra of the thiols listed. Comparing the spectra of the cyclic sulfides in Table IV to the data listed by Levy and Stahl for thiols and sulfides, one finds that m/e 43 is essentially absent in all of the spectra of the cyclic sulfides. For acyclic sulfides the m/e 43 ion is present in a relative abundance of five percent or greater in seventy two percent of the sulfide spectra and in seventy nine percent of the thiol spectra listed by Levy and

Stahl. From this data it would be safe to assume that the absence of an m/e 43 ion in the spectrum of a sulfur containing compound would support the existence of a cyclic structure, but would not be sufficient evidence alone. The absence of the m/e 43 ion in the spectra of the cyclic sulfides in Table IV does not seem to carry through to fused ring systems^{25,26}.

SCHEME VIII

 m/e 102

SCHEME VIII (con't.)



Comparing 2,2-dimethylthietane to the other cyclic sulfides, it is interesting to note that the base peaks for all of the sulfides except 2,2-dimethylthietane are due to the loss of either a methyl radical or two carbons from the ring with retention of the charge on the fragment containing the sulfur. 2,2-Dimethylthietane is the only compound in the series where the base peak is due to loss of a sulfur fragment with retention of the charge on the sulfur-free alkyl group. This difference in the base peaks can be explained by examining the

Table IV^a

	Mol. Wt.	M-34	Rel. int.	M-33	Rel. int.	Base peak	Fragment Lost
Thiacyclobutane	74	40	1.0	41	8.6	46	$\text{CH}_2=\text{CH}_2$
2-Methyl	88	54	18.0	55	25.0	60	$\text{CH}_2=\text{CH}_2$
3-Methyl	88	54	6.5	55	7.3	46	$\text{CH}_2=\text{CHCH}_3$
2,2-Dimethyl	102	68 ^b	18.4 ^b	69 ^b	32.3 ^b	56 ^b	$\text{CH}_2=\text{S}$
Thiacyclopentane	88	54	12.4	55	12.3	60	$\text{CH}_2=\text{CH}_2$
2-Methyl	102	68	6.0	69	3.5	87	CH_3
3-Methyl	102	68	21.1	69	24.1	60	$\text{CH}_2=\text{CHCH}_3$
2,5 Dimethyl cis or trans	116	82	2.0	83	1.0	101	CH_3
Thiacyclohexane	102	68	70.0	69	19.0	102,87	CH_3
2-Methyl	116	82	10.4	83	4.7	101	CH_3
3-Methyl	116	82	10.1	83	2.0	101	CH_3
4-Methyl	116	82	38.1	83	7.2	116,101	CH_3
Thiacycloheptane	116	82	42.9	83	7.6	87	CH_2CH_3

^a Unless otherwise referenced, data are from ref. 24^b Ref. 6

possible fragmentation pathways. The two competing pathways involve a radical site initiated mechanism and a charge site initiated mechanism for 2,2-dimethylthietane and the other cyclic sulfides. In the case of 2,2-dimethylthietane, a charged site initiated cleavage by the sulfur results in the migration of the electron pair of the 1-2 sulfur-carbon bond to the sulfur leaving a positive charge on the tertiary carbon. This is then followed by radical site initiated cleavage of the carbon-carbon bond alpha to the sulfur, as diagrammed in Scheme VIII. The other sulfides in Table IV cannot offer a tertiary carbon as an alternate charge site. This results in radical site initiated cleavage dominating the fragmentation in which the charge is retained on the fragment containing the sulfur.

The data from the remaining peaks in the gas chromatogram were not analyzed due to overlap of the data that could not be separated with the methods available.

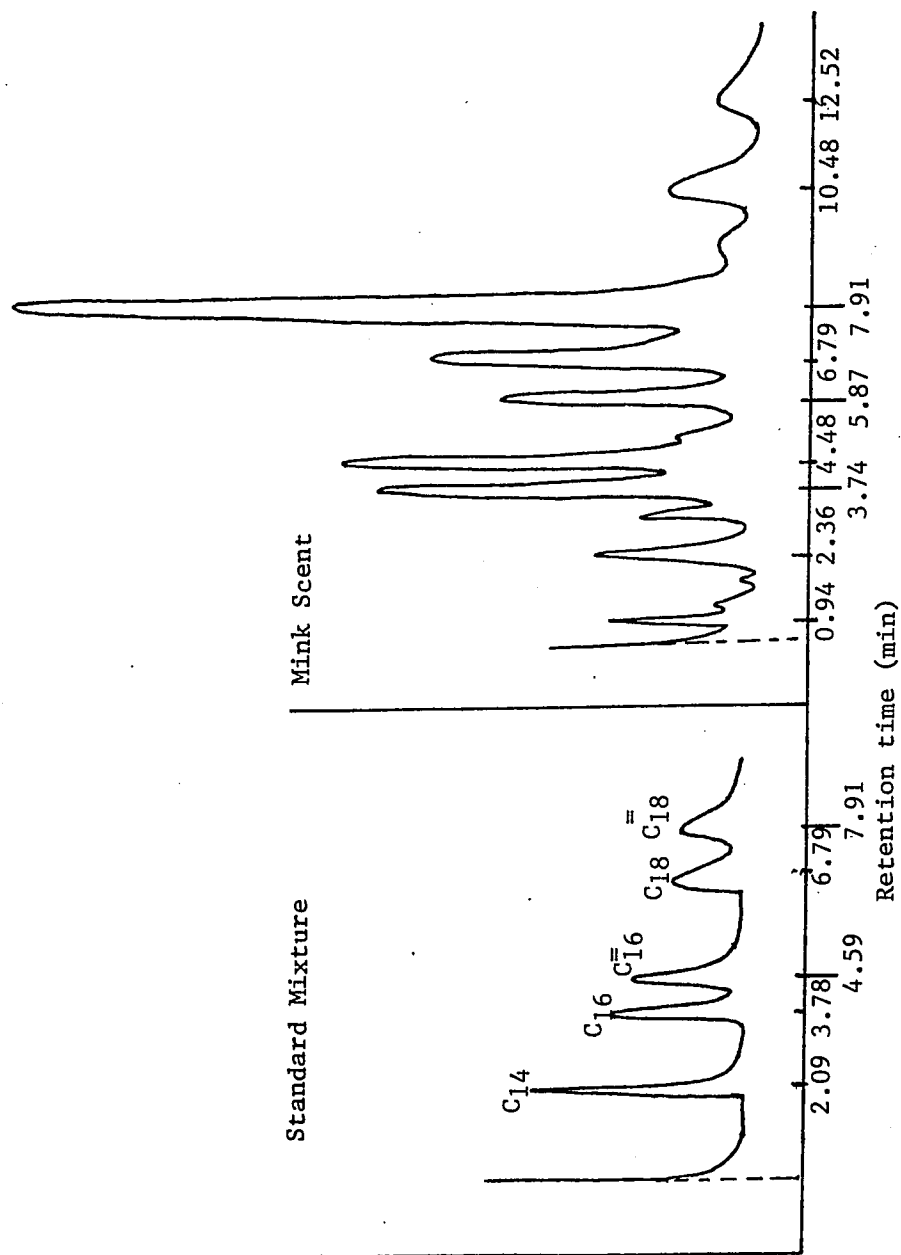
Nonvolatile Components of Mink Anal Sack Secretions

At the present time there is no published report of the chemical nature of the nonvolatile components of mink anal sack secretions. The nonvolatile portion is a fairly viscous straw colored oil about the same consistency as honey. Thin layer chromatography showed four major and four minor spots. The major spots were all of higher R_f values than the minor ones. The nmr of the total nonvolatiles (nmr spectra 26, 27) shows a very large methylene envelope at $\delta 1.3$, methyl signals at $\delta 0.9$, a pentet at $\delta 2.35$, a triplet at $\delta 4.16$, and vinyl protons at $\delta 5.47$. The infrared spectrum (1) shows a very strong sp^3 C-H stretching band at 2930 cm^{-1} , a strong C=O stretching band at 1640 cm^{-1} , C-O stretching at

1170 cm^{-1} , absence of any C-H out of plane bending, and a broad band at 700 cm^{-1} , which is indicative of methylene rocking in long chain molecules. This nmr and ir data is typical for triglycerides.

Column chromatography using a hexane to ether gradient provided two fractions that contained the two most intense spots of the previous tlc. These fractions were treated with boron trichloride in methanol to yield the methyl esters of the fatty acids of the triglycerides. Gas chromatographic analysis of the mixture of methyl esters with comparison to a standard mixture of methyl esters showed the largest peak in the gas chromatogram of the mink scent had the same retention time as the eighteen carbon chain with one double bond of the standard mixture. There were a total of fourteen peaks in the chromatogram of the mink scent, (see Diagram IV). Peak six had the same retention time as the sixteen carbon saturated chain. Only a tentative assignment can be made on the basis of retention time alone. Further analysis of the mixture requires capillary column separation coupled to a mass spectrometer. This instrumentation was not available for this project.

DIAGRAM IV



Gas chromatograms of fatty acid methyl esters from mink triglycerides, and a standard mixture of fatty acid methyl esters.

SUMMARY

The previous work dealing with the chemical composition of skunk anal sack secretions showed trans-2-butene-1-thiol, 3-methyl-1-butanethiol and crotyl methyl disulfide to be the major constituents. The present work found 1-butanethiol, crotyl isopentyl sulfide, dicrotyl sulfide, crotyl propyl sulfide, crotyl isopentyl disulfide, and dicrotyl disulfide as minor components in the volatile portion of the anal sack fluid. The three previously found components were evident in the nuclear magnetic resonance spectrum of the total fluid, but only 3-methyl-1-butanethiol was not obscured during the mass spectral analysis of the minor components.

Chromatographic separation of the non-volatile portion of skunk anal sack fluid indicates a minimum of thirteen compounds. Analysis of the fractions by ultraviolet and nuclear magnetic resonance spectroscopy shows that the majority of the compounds are aromatic. Separation of two of the compounds and further analysis by chemical and spectroscopic methods showed the 2,6-disubstituted quinoline structure to be a possibility.

Analysis of the mink anal sack fluid by nuclear magnetic resonance spectroscopy showed 2,2-dimethylthietane and diisopentyl disulfide to be present. Previous work by Schildknecht showed 3,3-dimethyl 1,2-dithiolane to be present, but this was not found in this study. The nonvolatile portion of mink anal sack fluid was comprised of a mixture of triglycerides. The relative amounts of volatiles to non-volatiles was much greater in the skunk fluid than in the mink fluid.

CONCLUSION

The previously reported work on the anal sack secretions of the striped skunk identified the major constituents of the scent as 3-methyl-1-butanethiol, 2-butene-1-thiol and crotyl methyl disulfide. In this present study the nmr of the total oily phase (nmr spectrum 30) of the scent shows the presence of these compounds in large quantities relative to all other components that are visible in the spectrum. Mass spectral analysis of the scent does not show the presence of either of the crotyl compounds that are obvious in the nmr, although other crotyl compounds that are not apparent in the nmr are identified in the mass spectra. This can be explained by the gas chromatogram. In the gas chromatogram of the total oil that was used for the mass spectral analysis, the two compounds that don't appear are overlapped by other compounds that are not as well resolved; therefore, the overlapping compounds have a broader base. The large excess of the "missing" crotyl compounds would have flooded the ionization chamber of the mass spectrometer, so as these compounds eluted from the gas chromatograph they were diverted out of the flow to the mass spectrometer. Cutting the peaks at the leading and tailing edge afforded spectra of the overlapping compounds with the broader base but not of the crotyl compounds.

The volatile compounds isolated from the mink also proved to be sulfur compounds, although the only thing in common with the volatiles from skunk scent is the isopentyl moiety. The relative amount of volatile to nonvolatile is significantly different between

the skunk and the mink. With the majority of skunk scent volatile, and the majority of mink scent nonvolatile, it becomes apparent that even though the two animals belong to the same family, the highly developed secretion system that they both possess is used for two entirely different purposes. Also, the nonvolatile components are as important as the volatile ones. The outstanding difference between the nonvolatile components is not their different relative abundances. That could have been logically deduced from the known behavior of each animal. It is the chemical differences. The skunk produces an unstable nitrogen containing, aromatic mixture, whereas the mink produces a stable mixture of triglycerides. Each nonvolatile mixture is chemically suited for the intended use. The skunk uses its scent primarily for defense. The effect of the scent has to be instantaneous and strong, but it doesn't have to be effective over a long period of time. The nonvolatile portion acts as a carrier for the effective part of the scent. The mink uses its scent primarily for territorial marking, not for defense. In this case the scent has to be noticeable, but not on the same order of magnitude as the skunk's scent. It is more important that the scent be effective for a long period of time.

The chemical taxonomy of animals helps to fill important pieces of the picture of how these animals interact with their surroundings. Laboratory studies of animal behavior and the chemical understanding of the composition and function of animal scents has resulted in efforts to apply this knowledge to wildlife management problems³¹.

EXPERIMENTAL

The nuclear magnetic resonance spectra were obtained using a JEOL MH-100 nuclear magnetic resonance spectrometer. The infrared absorption spectra were obtained using a Perkin-Elmer model 337 grating infrared spectrophotometer. The ultra-violet spectra were obtained using a Bausch and Lomb Model 505 spectrophotometer. The boiling points and melting points are uncorrected and are in degrees Celsius. The gas chromatographic analyses were achieved using an Aerograph A-90-P3 gas chromatograph. The mass spectra were obtained using a modified Hitachi RMU-6 spectrometer, computer assisted.

Mink Anal Sack Secretion

Procurement of the Anal Sack Secretion. The anal sack secretions were collected from male and female mink during November 1974 and November 1975. The mink had been dead and skinned for approximately 48 hours in the case of the samples collected in 1974, but only for a few hours for the samples collected in 1975. The fluid was removed by syringe from the anal scent sacks by piercing the sack with a twenty gauge needle, and slowly withdrawing the fluid. The scent sacks were located on either side of the body midline on the ventral side of the body between the anus and the base of the tail. The sacks varied in size from 1 to 1.5 cm in length and 0.5 cm in width. The fluid was a thick off-white emulsion that had a very strong, unpleasant odor of sulfur compounds. The secretion volume averaged about 1.25 ml, with the males usually containing more, simply because of their larger body size. The samples were taken from fifty to sixty individuals each

year. The secretions from the males and females were kept separate and immediately frozen.

Initial Separation by Centrifugation. The total scent from either the male or the female mink was centrifuged for approximately two hours, resulting in the formation of four distinct layers. The top layer was a light yellow oil that comprised 20% of the total scent in the case of the females and 34% in the case of the males. A thick whitish fatty suspension at the interface of the top oil layer and the lower aqueous layer comprised the second layer. The aqueous layer was pale yellow. The layer below the aqueous layer was similar in color and appearance to the fatty suspension, but of about half the volume. The top oil layer was drawn off and frozen, as was the remainder of the scent.

Separation of the Volatiles from the Nonvolatiles. A small flask containing the total oil phase was deoxygenated by displacing the air with nitrogen. The flask was then attached to the distillation apparatus, which also had been flushed thoroughly with nitrogen, and then closed to the atmosphere. With the entire system closed to the atmosphere, the flask containing the total scent was immersed in a liquid nitrogen bath. The above procedure was necessary to keep oxygen from liquifying in the sample flask during freezing. Once the sample was frozen, the system and sample flask was evacuated to 0.02 mm (with stopcocks 1, 2, 3, 4 and 6 open; 5 closed, see Diagram V). When 0.2 mm was attained, stopcocks 1 and 4 were closed. The liquid nitrogen bath was removed from the sample flask and placed around the condensing flask. After the sample flask had warmed to room temperature, it was heated to 40°C with a water bath. Approximately 0.2 ml of a color-

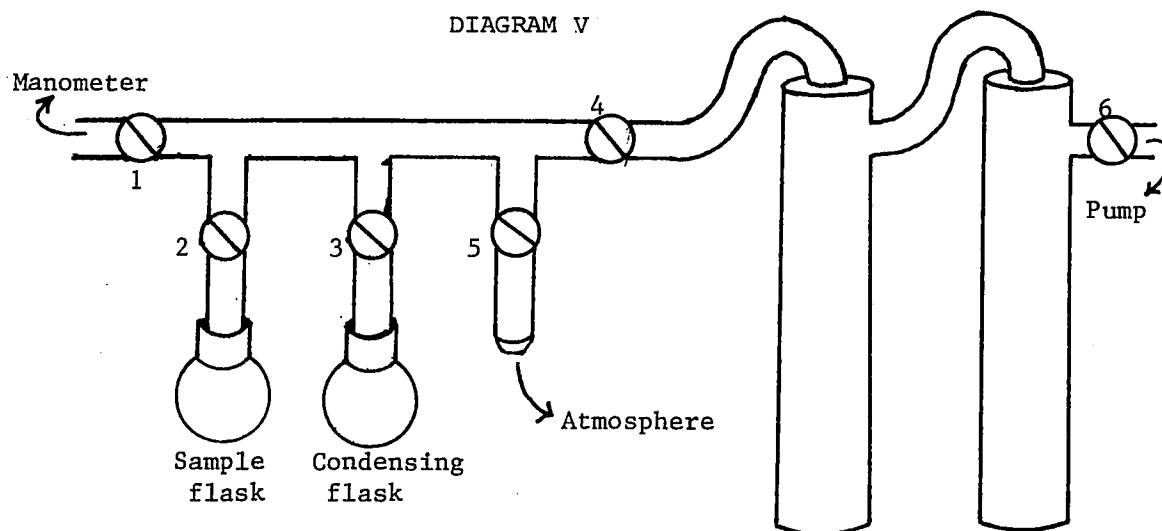
less liquid that had strong unpleasant sulfide- or thiol-like smell condensed in the cooled flask.

Preparative Gas Chromatography of the Volatiles. The volatiles were separated by gas chromatography under the following conditions.

Column : Teflon-lined aluminum : length, 12 ft. : 0.25 in O.D. :

Chromosorb-W 60/80 mesh : Apiezon-L 20% : Temperature 150 - 230°C :

Peak retention times 4:32, 18:57 (min:sec).



Gas Chromatographic Analysis of the Volatiles. The volatile portion of the total oil was compared to known compounds. MS designates mink scent.

Run #	Compound	Retention time (min:sec)	Temp. (°C)
a ₁	MS	9:15	80
a ₂	3-methyl-1-butanethiol	9:49	80
a ₃	MS+3-methyl-1-butanethiol	8:49, 9:23	82
a ₄	MS+1-butanethiol	5:53, 8.53	82
a ₅	MS	3:41	99
a ₆	3-methyl-1-butanethiol	3:45	99
a ₇	isopentyl methyl disulfide	29.04	99
a ₈	2-butene-1-thiol	2:41	99
b ₉	MS	5:07, 22:57	150-230
b ₁₀	MS	5:11, 23:00	150-230
b ₁₁	2,2-dimethylthietane (22DMT)	5:06	150-230
b ₁₂	diisopentyl disulfide (DID)	22:55	150-230
b ₁₃	MS 22DMT DID	5:08, 22:56	150-230
c ₁₄	2,2-dimethylthietane	2:13	150-230
c ₁₅	2,2-dimethylthietane	2:17	150-230
c ₁₆	diisopentyl disulfide	10:41	150-230
c ₁₇	diisopentyl disulfide	10:23	150-230
c ₁₈	3-methyl-1-butanethiol	1:41	150-230
c,d ₁₉	total oil phase	1:41	
	trace	5:11, 8:23, 10:49, 11:26	150-230

^a Apiezon-L 5%, Chromosorb W 60/80, column 12 ft X 0.25 in

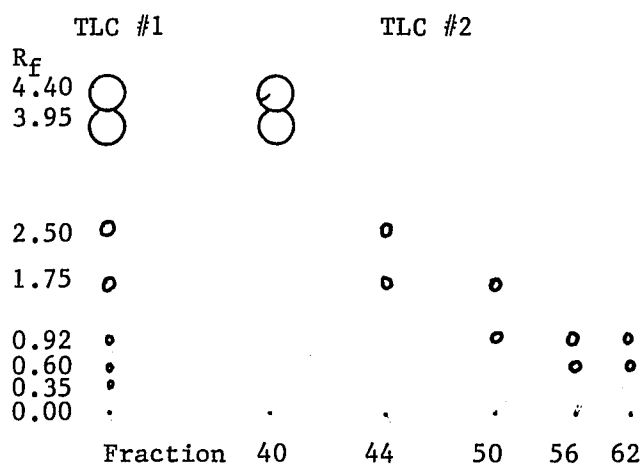
^b Apiezon-L 25%, Chromosorb W 60/80, column 12 ft X 0.25 in

^c Apiezon-L 25%, Chromosorb W 60/80, column 5 ft X 0.25 in

^d This sample of mink scent was stored at room temperature for six months.

Chromatographic Analysis of the Nonvolatiles. The nonvolatile portion of the anal sack secretion was subjected to many analyses by thin layer chromatography using a myriad of solvent systems. The best system was found to be a hexane ether mixture in a ratio of 9:1. TLC in Diagram VI was representative of the results.

Diagram VI



The nonvolatile material was then subjected to column chromatography. A gradient of 100% hexane to hexane/ether 1/3 on Mallinckrodt SilicAR cc-7, 100-200 mesh, column length 3 ft width 0.5 I.D. was used to elute the sample. One hundred fractions (10 ml) were collected and tlc'd. Selected fractions are shown in TLC #2 in Diagram VI. Fractions 39 and 40 from the column chromatography were combined and were chromatographed using hexane/ether 9:1 as the eluant. Twenty fractions were collected, with fractions 7-10 containing the desired compounds. Tlc analysis of each fraction showed fraction 7 to contain only one spot, fractions 8 and 9 contained two spots and fraction 10 contained only one spot. Considering that the nonvolatiles appear to be a mixture of triglycerides, the probability that the single tlc spots contain only one compound is

very low.

Proton Magnetic Resonance Spectra of Nonvolatiles.

See appendix

Total nonvolatiles nmr 26 and 27

Column chromatography II, fraction 7, nmr 28

Column chromatography II, fraction 10, nmr 29

Infrared spectra of nonvolatiles. See appendix.

Total nonvolatiles, IR 1

Column chromatography II, fraction 7, IR2

Column chromatography II, fraction 10, IR 3

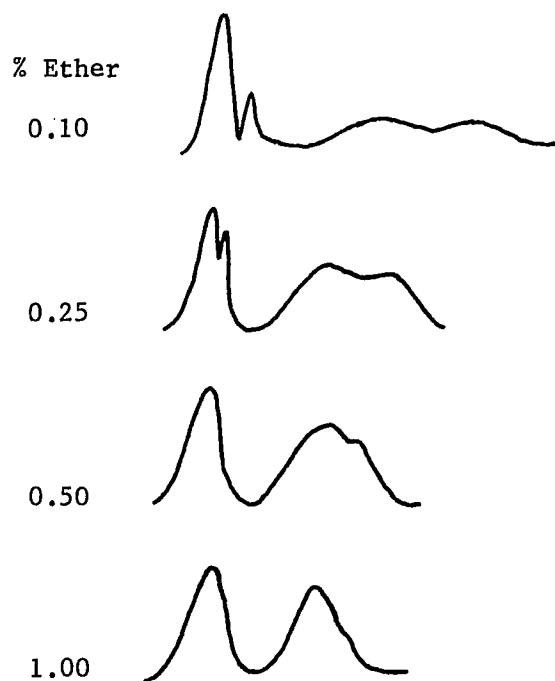
High Pressure Liquid Chromatography of Fractions 39 and 40.

Fractions 39 and 40 were identical by tlc. A portion of fractions 39 and 40 were analyzed by HPLC under the following conditions: stationary phase - Corisil 2, column dimensions - 2 ft x 1/8 in I.D., solvent - hexane/ether, two columns in series. A solvent ratio of hexane/ether 99.9/0.1 resolved the two spots from the column chromatography into four peaks on HPLC. The resolution on HPLC was very sensitive to the concentration of ether. Concentration changes of 0.05% resulted in significant changes in the resolution (see Diagram VII).

Transesterification of the Triglycerides in Fractions 39 and 40.

A few milligrams of fractions 39 and 40 of column chromatogram I of the nonvolatiles were dissolved in absolute methanol (5 ml) and placed in a 100 ml round bottom flask. Boron trichloride was bubbled through the solution at a rate of 100 bubbles per minute for five minutes, with stirring. The solution was then refluxed for ten minutes. The solvent was stripped off, and the resulting oil was taken up in purified methylene chloride. The methylene chloride solution was subjected to G.C. analysis.

DIAGRAM VII
HPLC of Fractions 39 and 40 (mink non-volatiles)



Gas Chromatographic Analysis of the Transesterification Products.

The samples were analyzed as methylene chloride solutions under conditions as follows: 12% diethyleneglycol succinate on Anakrom A, column dimensions - 6 ft X 0.25 in OD, column temperature, 200°C.

Standard known mixture K102 lot #2686

Methyl mystrate 20% (C₁₄)

Methyl palmitate 20% (C₁₆)

Methyl palmitoleate 20% (C₁₆)

Methyl oleate 20% (C₁₈)

Methyl stearate 20% (C₁₈)

Standard mixture - retention times measured from methylene chloride peak.

Compound	Retention Time (min:sec)
C ₁₄	1:23
C ₁₆	2:31
C ₁₆ ⁼	3:04
C ₁₈	4:32
C ₁₈ ⁼	5:17

Unknown mixture - retention times were measured from the methylene chloride peak.^a

Compound	Retention Time (min:sec)
1	0:38
2	1:09
3	1:13
4	1:34
5	2:08
6	2:30
7	3:00
8	3:21
9	3:55
10	4:32
11	5:17
12	6:05
13	7:00
14	8:21

^aSee Diagram IV for the actual chromatograms.

Preparation of 1,3-Dichloro 3-Methylbutane²⁷. Isoprene

(53.0g, 0.78m) was slowly added to 270 ml of concentrated hydrochloric acid with vigorous stirring at room temperature. The reaction was allowed to stir for 24 hours after the addition was complete. The organic layer was isolated and washed with 5% NaHCO_3 . The organic layer was taken up in methylene chloride and dried over Na_2SO_4 . Removal of the solvent by slow distillation gave 71.2g, 65.2% yld.

Preparation of 2,2-Dimethylthietane. 1,3-Dichloro-3-methylbutane (40.31g, 0.28m) was placed in a 500 ml 3 neck flask fitted with a reflux condenser and two gas inlet tubes. Hydrogen sulfide (dry and chemically pure-MCB) was bubbled through the dichloride for 30 minutes. Then, approximately 2 cc of anhydrous aluminum chloride was added to the solution. Immediately the reaction mixture turned bright reddish-orange²⁸. If all of the aluminum chloride was added at once, the reaction mixture foamed into the condenser. Hydrogen sulfide was again bubbled through the mixture this time for 20 minutes. The mixture was allowed to stand for 20 minutes. Then 1 cc of anhydrous aluminum chloride was added and hydrogen sulfide was bubbled through again. This process was repeated five more times. The reaction mixture was then allowed to stand for six hours. The reaction mixture was then cooled to -10°C , poured into a solution of 40% NaOH at -10°C , and stirred for 10 minutes. The resulting emulsion was extracted with ether. The ether solution was dried over anhydrous MgSO_4 . The product was recovered by distillation. Yield 15.3g, 52.6%, bp 55°C , (100 mm), nmr 23, lit. bp 115°C ²⁸.

Preparation of 3-Methyl-1-butanethiol. 3-Methyl-1-bromobutane (18.92g, 0.13m) and thiourea (9.88g, 0.13m) in 100 ml of 95% ethanol²⁹ was heated at reflux for 3 hours. The solvent was removed in vacuo,

150 ml of 20% KOH was added and the resulting solution was stirred for 2 hours. After acidification with concentrated hydrochloric acid, the organic layer was separated, dried over anhydrous MgSO_4 , and distilled to yield 7.61g, 56.2%, bp 67°C , (200 mm), lit. bp 118°C ³².

Preparation of Diisopentyl Disulfide. 3-Methyl-1-butanethiol (6.36g, 0.061m) was stirred at 90°C for three hours with an excess of dimethyl sulfoxide³⁰. The reaction mixture was cooled, 300 ml of water was added and the resulting aqueous solution was extracted with two 30 ml portions of methylene chloride. The organic layer was dried over anhydrous MgSO_4 and concentrated by distillation to give 2.74g of crude product, 43.6% yield (nmr 25).

Skunk Anal Sack Secretion

Procurement of the Anal Sack Secretions. Anal sack secretion or scent was obtained from five skunks. Two of the skunks were males that were found freshly killed along the road in the vicinity of the town of Durham, New Hampshire. The other three skunks were females. Two of the females were captured alive - one in Portsmouth and one in Durham - the third was shot in Milton, New Hampshire. The female captured in Portsmouth was lactating at the time the scent was removed. Both of the captured skunks were anesthetized with ether while the scent was removed with a blunt tip needle and syringe by entering the anal sacks through the ducts used by the skunk for spraying the scent. The scent was then immediately frozen. Scent from the dead skunks was obtained in the same manner. The sacks were located in the same area of the body as the mink anal sacks, but were approximately three times as large as the mink anal sack. Between 4 and 5 ml of fluid, which immediately separated into two phases with a whitish suspension in each

layer, was found in the anal sacks.

Initial Separation by Centrifugation. The total anal sack secretion separated into three layers upon centrifugation for two hours. A nonviscous amber colored oil that had a very strong mercaptan-like smell formed the top layer. The second layer appeared to be a whitish fatty suspension similar to the one found in mink scent. The bottom layer was a slightly yellow aqueous layer. The top layer comprised 40% to 65% of the total scent depending on the sample.

Separation of the Volatiles from the Nonvolatiles. A small flask containing the total oil phase was flushed with nitrogen and attached to the distillation apparatus outlined in Diagram V'. The distillation apparatus was also flushed with nitrogen and then sealed from the atmosphere. The scent was then frozen with liquid nitrogen and the system was evacuated to 0.2 mmHg. Stopcocks 1 and 4 were closed and the flask containing the scent was allowed to warm to room temperature and was heated to 80°C while the collection flask was immersed in liquid nitrogen. When the distillation was complete, a clear colorless liquid with a very strong mercaptan smell that comprised about 2/3 of the total scent was obtained. The residue, that was now a dark amber, was almost odorless. After the residue has been stored for a short time, the strong mercaptan-like smell returned.

Chromatographic Separation of the Nonvolatiles. The nonvolatiles that originated from the trap to trap distillation of the total oil were subjected to column chromatography under the following conditions: Silica gel 70-230 mesh, column dimensions 1.5 ft. X 5/16 in ID, eluant chloroform 100%. Approximately 0.4 ml of sample used. Fifty fractions were collected and examined by tlc, and an nmr was ob-

tained of each fraction. See appendix for selected nmr, 1-13. The tlc of selected fractions are given in Diagram II.

Preparative TLC of the Nonvolatiles. The nonvolatiles were examined by tlc using several solvent systems varying in composition from 100% chloroform to chloroform/ether 75^v/25^v. The gradual change resulted in all of the spots at R_f 0.4 or greater converging to two spots at R_f 0.65 and R_f 0.74. Spots below R_f 0.4 moved to higher R_f values accompanied by an increase in their resolution. These conditions were then transferred to preparative tlc and the compounds corresponding to R_f 0.54 and R_f 0.38 were isolated.

R_f 0.54 nmr 14, uv 1 and 2

R_f 0.38 nmr 15, uv 3 and 4

Gas Chromatographic Comparison of Individual Skunks. The samples used were the total oils from the skunks. G.C. conditions were as follows: column dimensions, 12 ft X 0.25 in OD; 20% on Carbowax 20M, Chromosorb W, 60/80 mesh; column T, 100°C; See appendix for actual gas chromatograms. Male 1, glc 1; male 2, glc 2; female (shot), glc 3; female (nonlactating), glc 4; female (lactating), glc 5.

Mass Spectral Analysis of the Volatiles. The gas chromatographic conditions worked out for the glc-mass spectrum run were as follows: column dimensions, 10 ft X 1/8 in OD stainless steel; 5% Carbowax 1500 on 120/140 mesh; Chromosorb W-(DMCS treated), column temperature, 50 to 120°C at 2.5°C/min; flow rate 30 ml/min. G.C. 6 was run on an independent gas chromatograph just prior to running the sample on the glc-mass spectrometer. The column was conditioned at 180°C with a flow of 10 ml/min for 36 hours. The glc-mass spectrometer used was a modified Hitachi MU-6, computer assisted, located at Massachusetts Insti-

tute of Technology. The glc trace for the glc-mass spectrometer run is g.c. 7. The corresponding total ionization intensity plot is given in Diagram I. One spectrum was obtained every 3 seconds for the duration of the gas chromatogram.

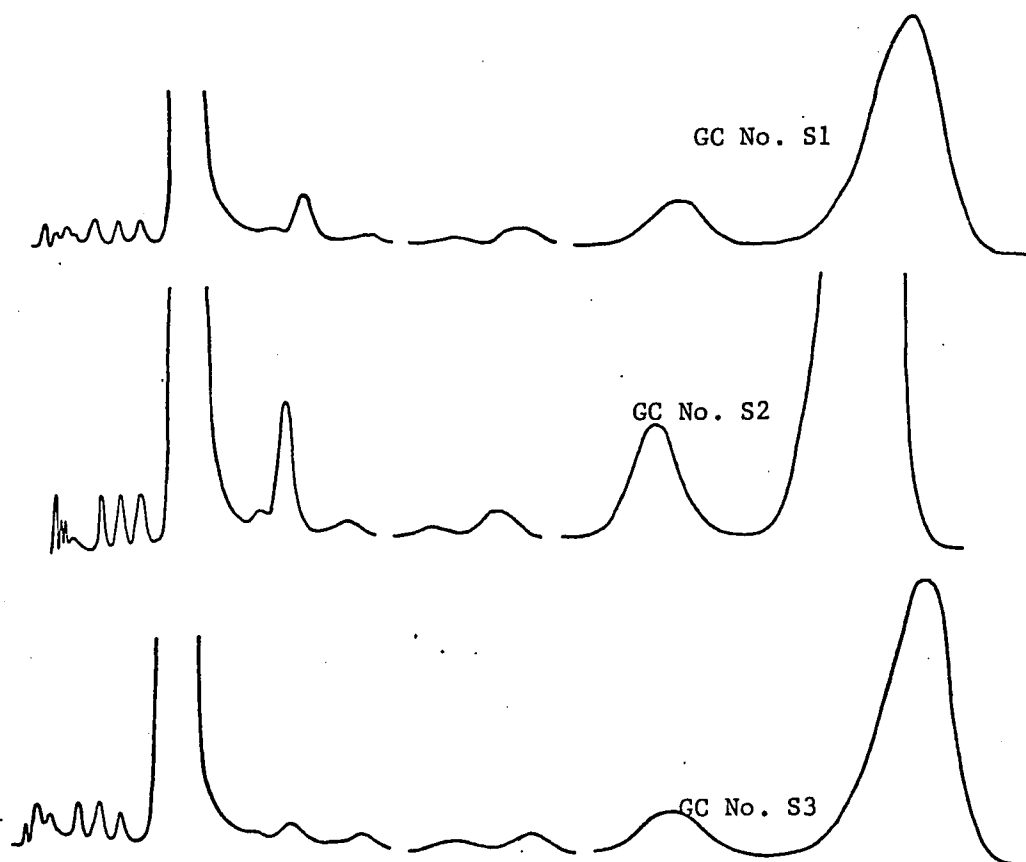
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APPENDICES

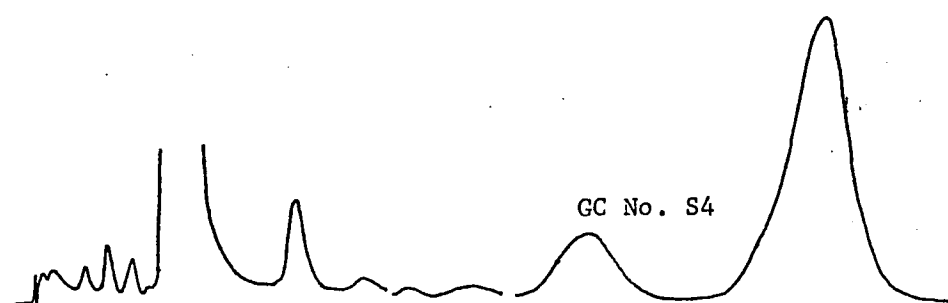
Gas Chromatograms
Skunk - Total Oil Phase



S1 - Male 1; S2 - Male 2; S3 - Female (shot).

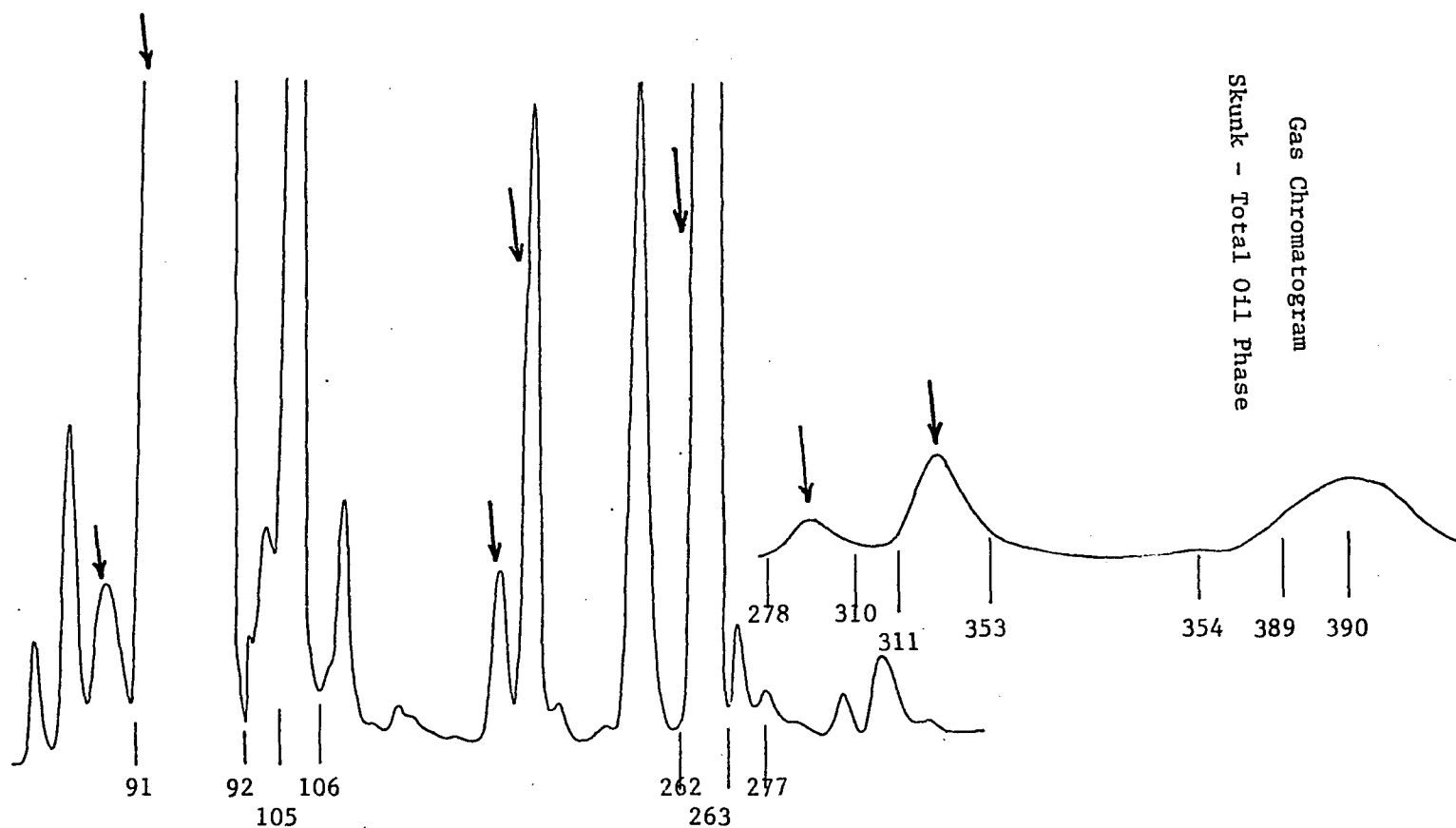
Gas Chromatograms

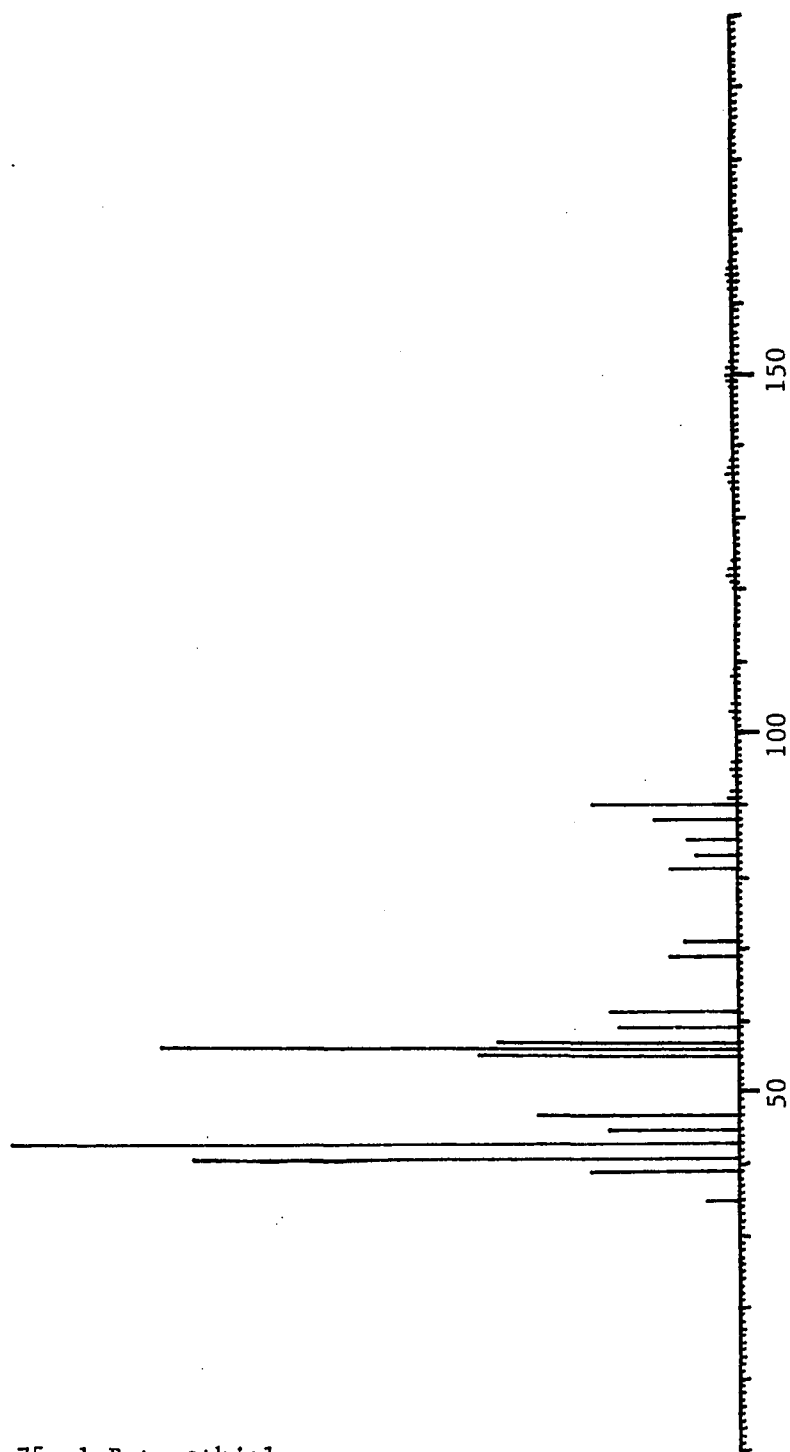
Skunk - Total Oil Phase



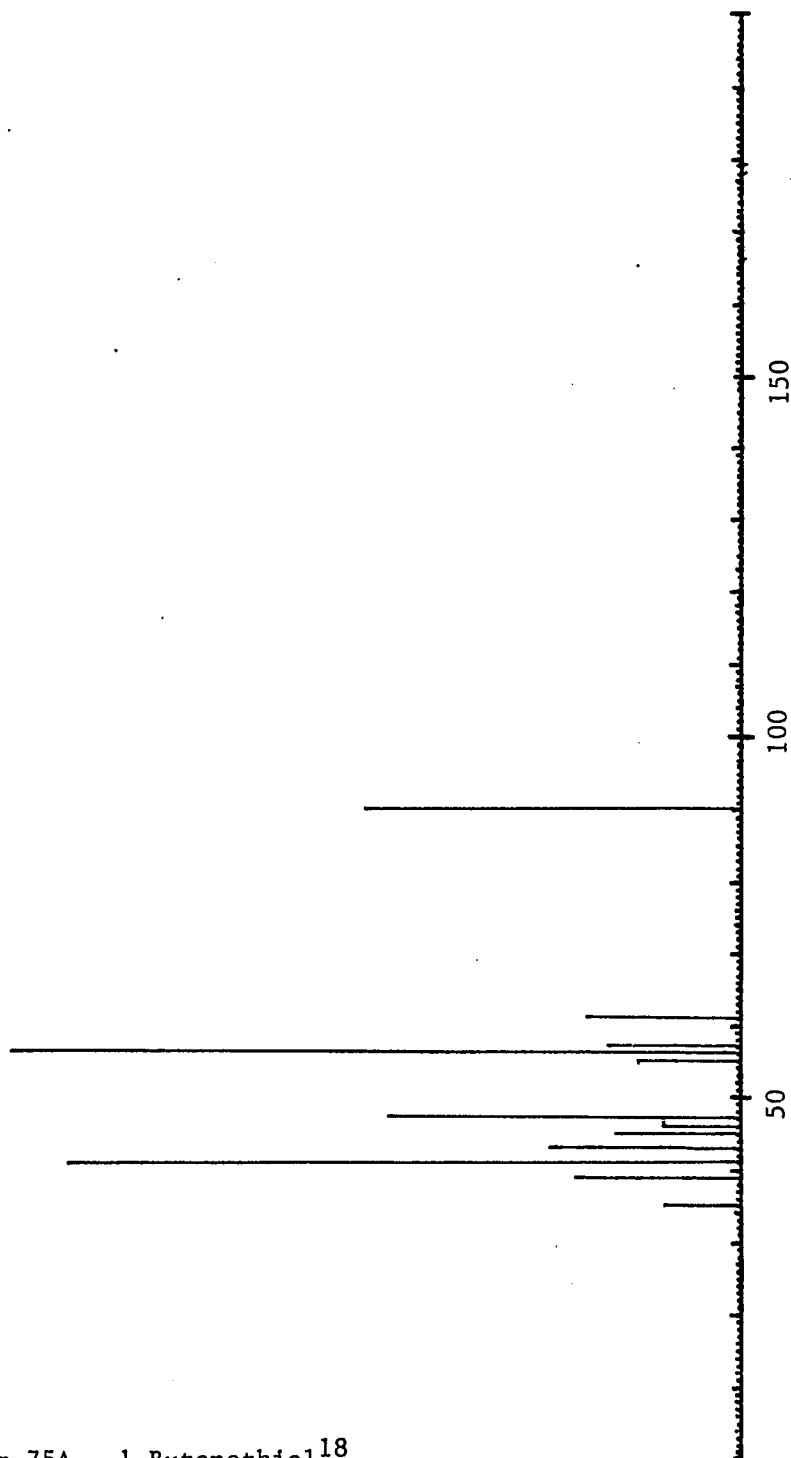
S4 - Female (nonlactating); S5 - Female (lactating).

GC No. S6,7; Carbowax 1500

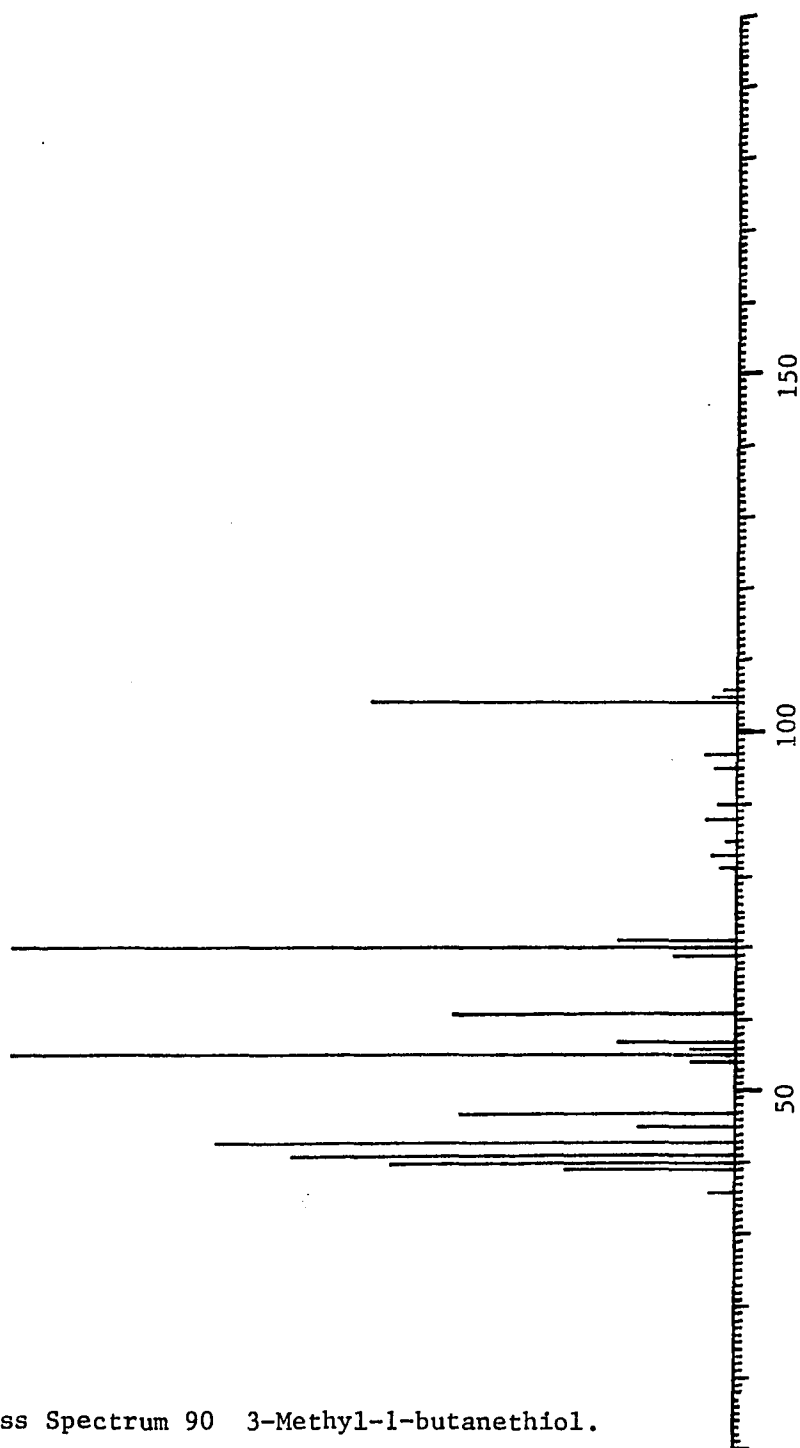


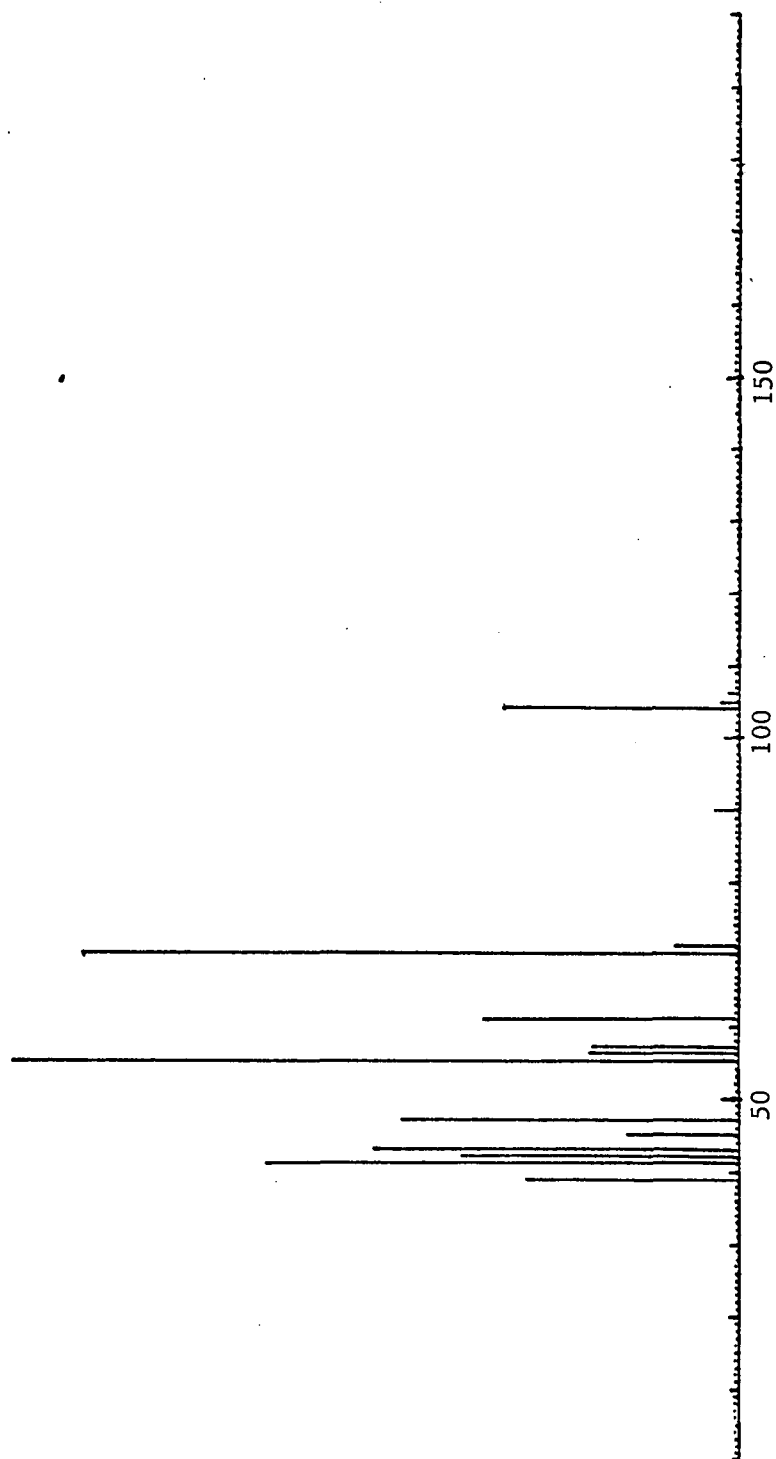


Mass Spectrum 75 1-Butanethiol.

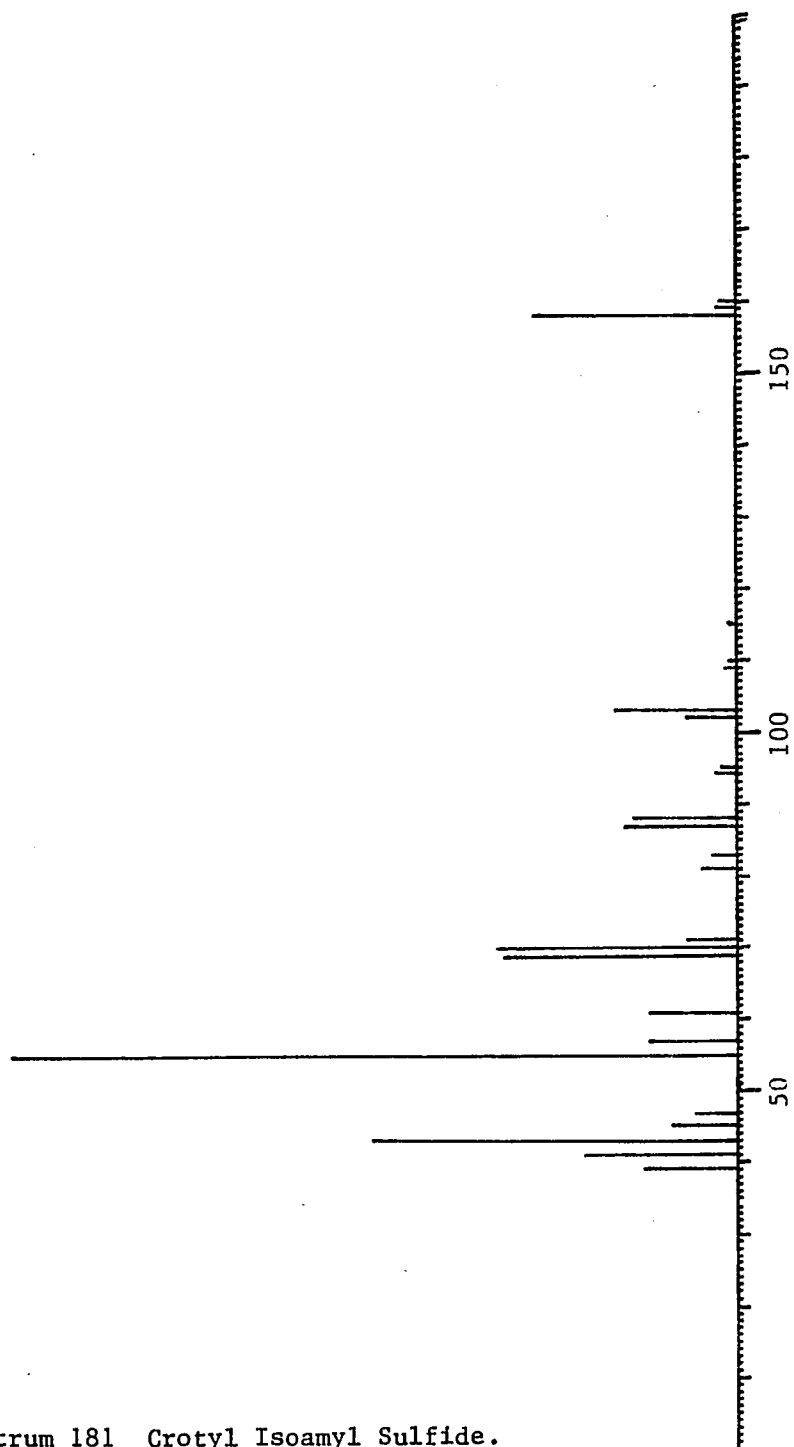


Mass Spectrum 75A 1-Butanethiol¹⁸

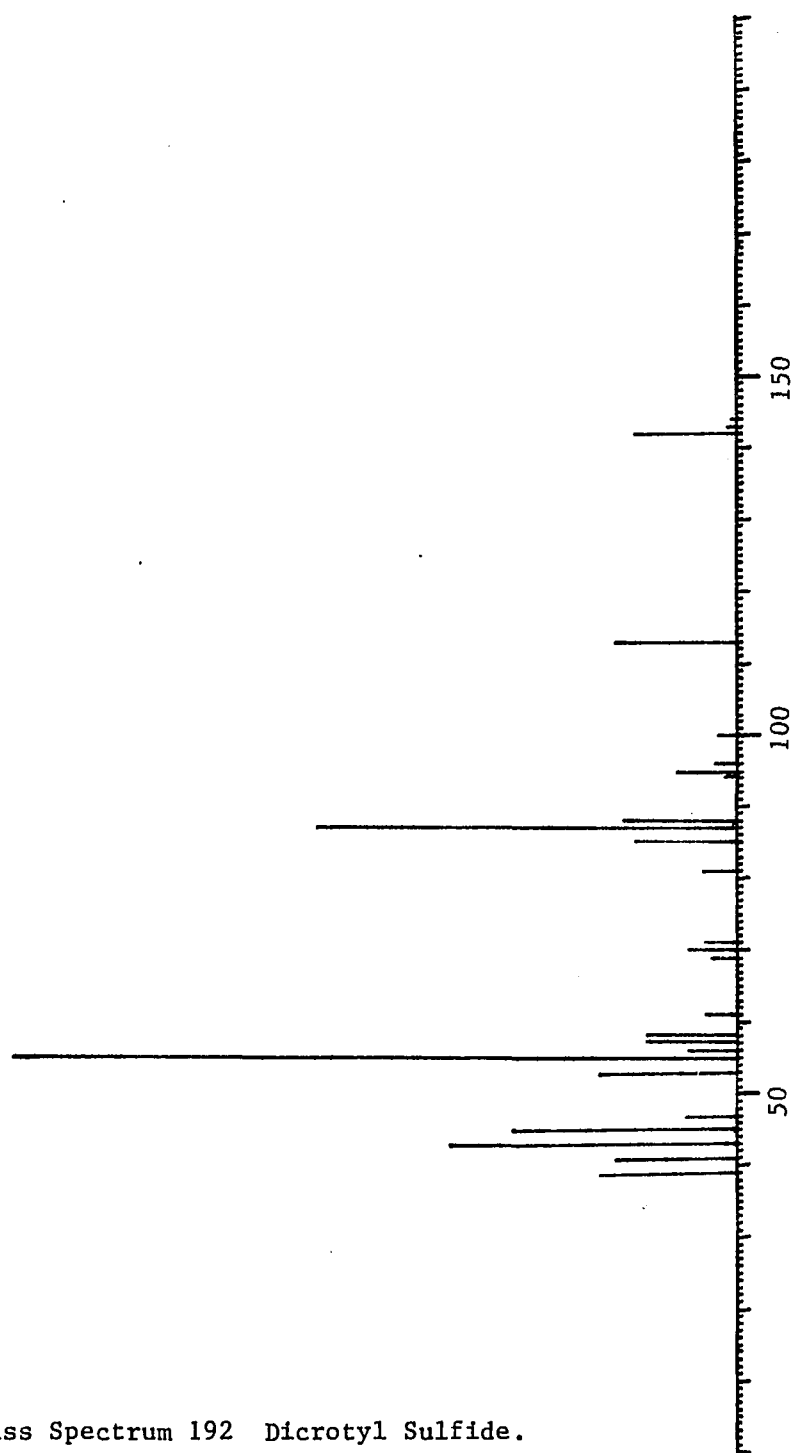


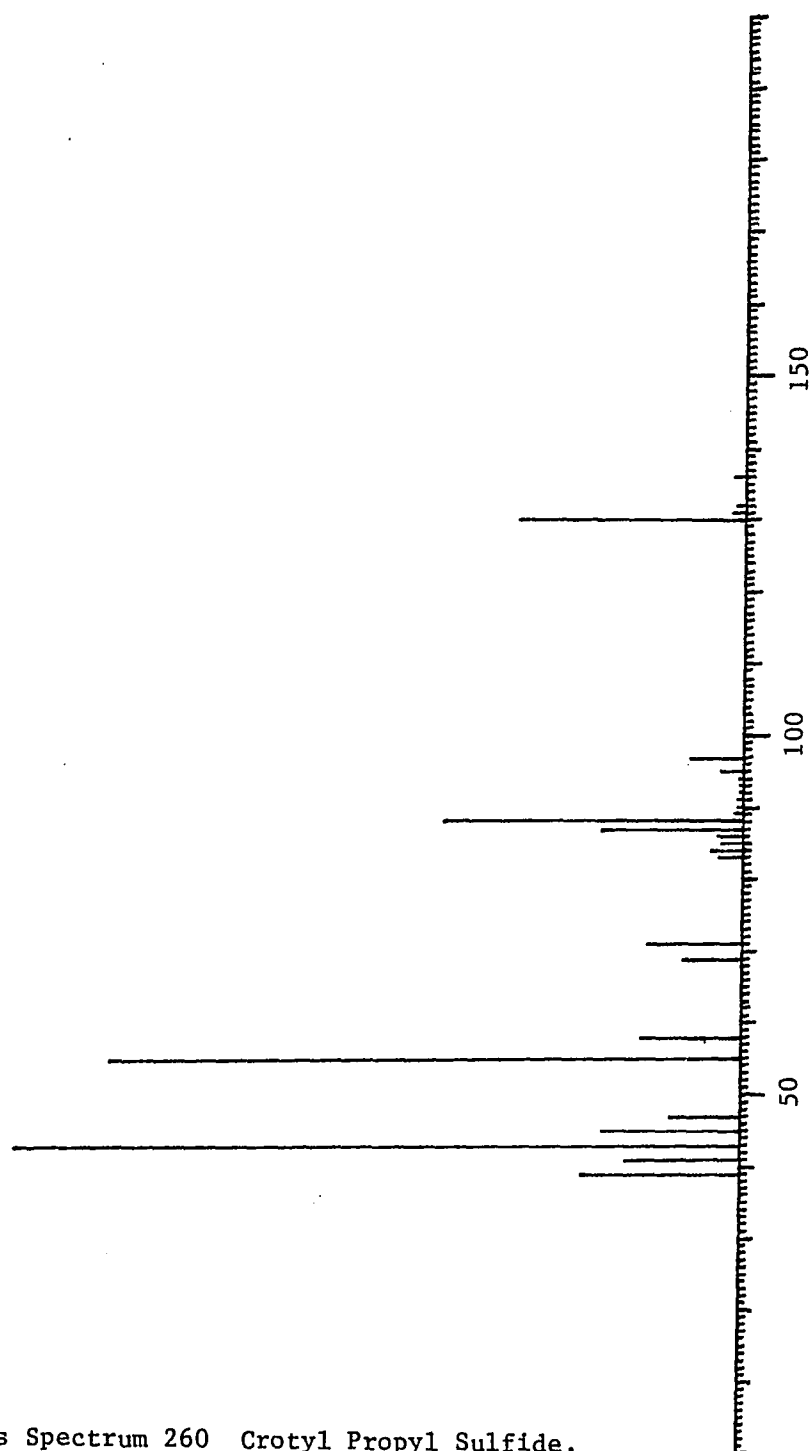


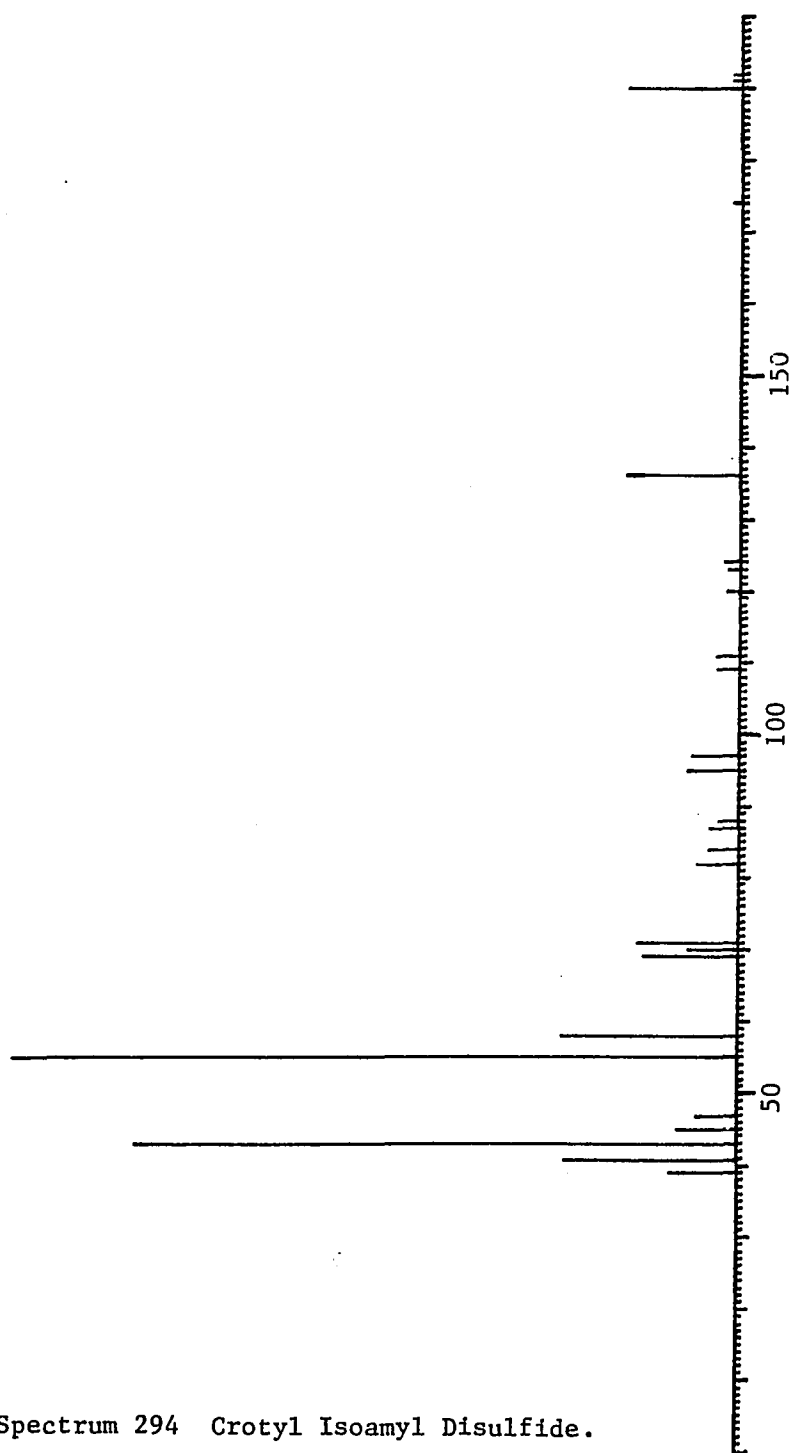
Mass Spectrum 90A 3-Methyl-1-butanethiol¹⁸.



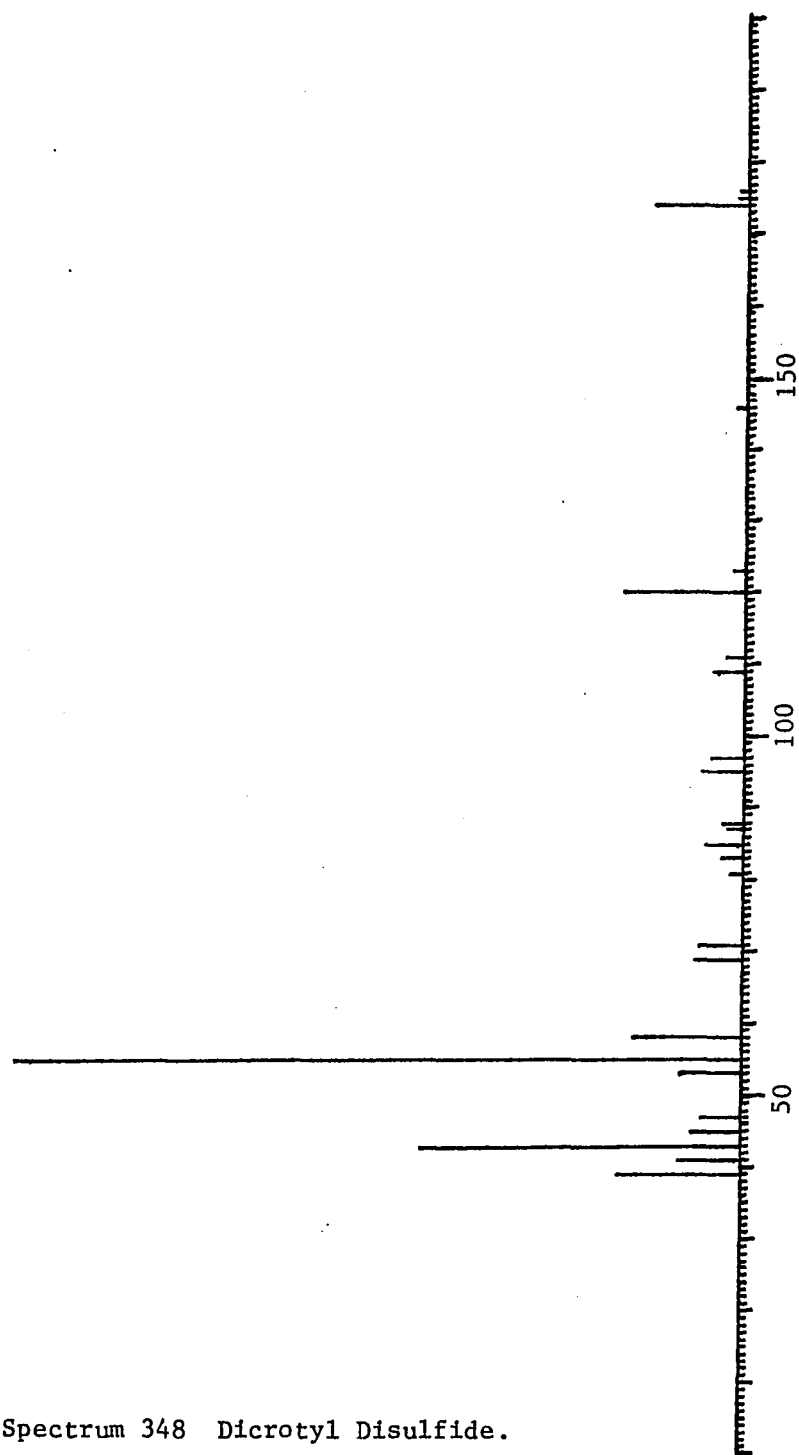
Mass Spectrum 181 Crotyl Isoamyl Sulfide.



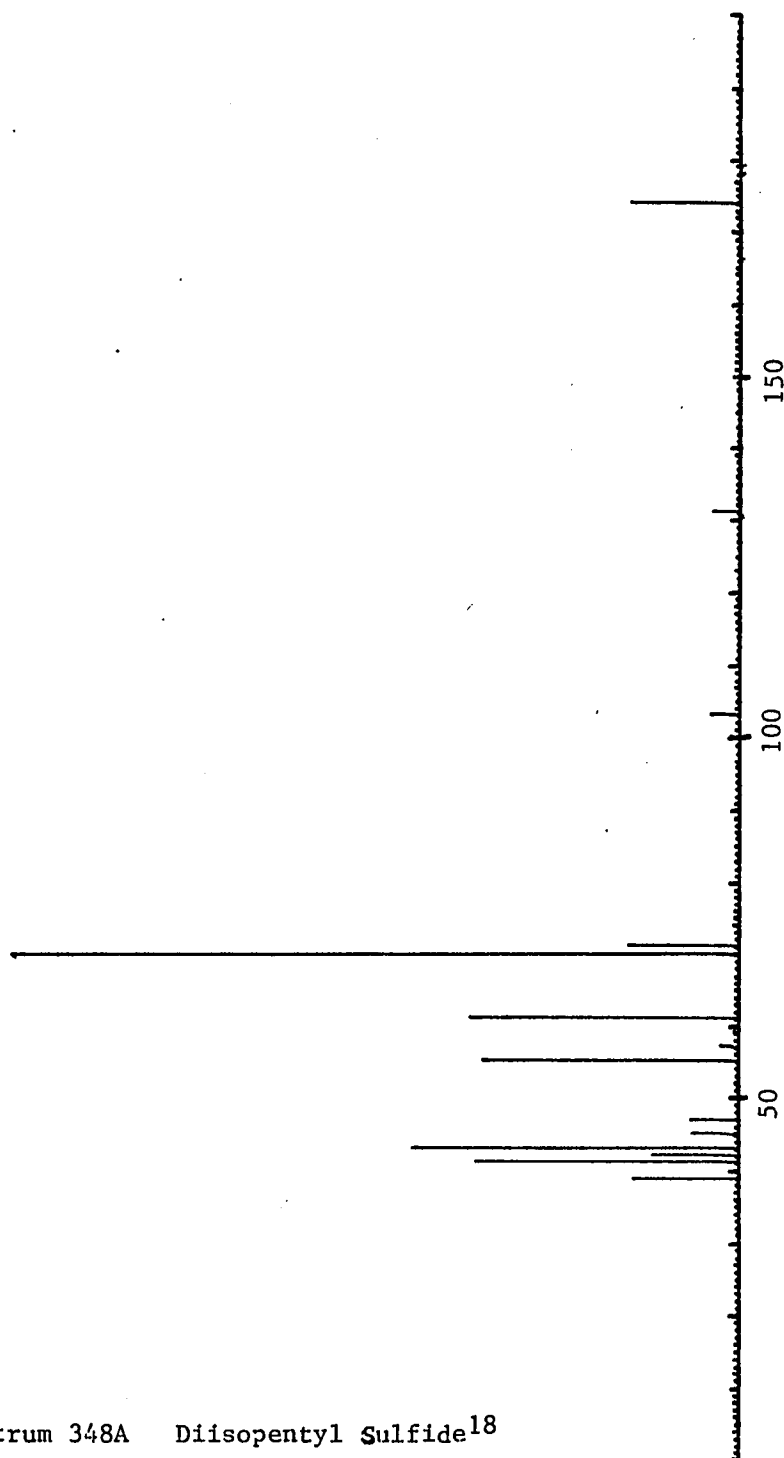




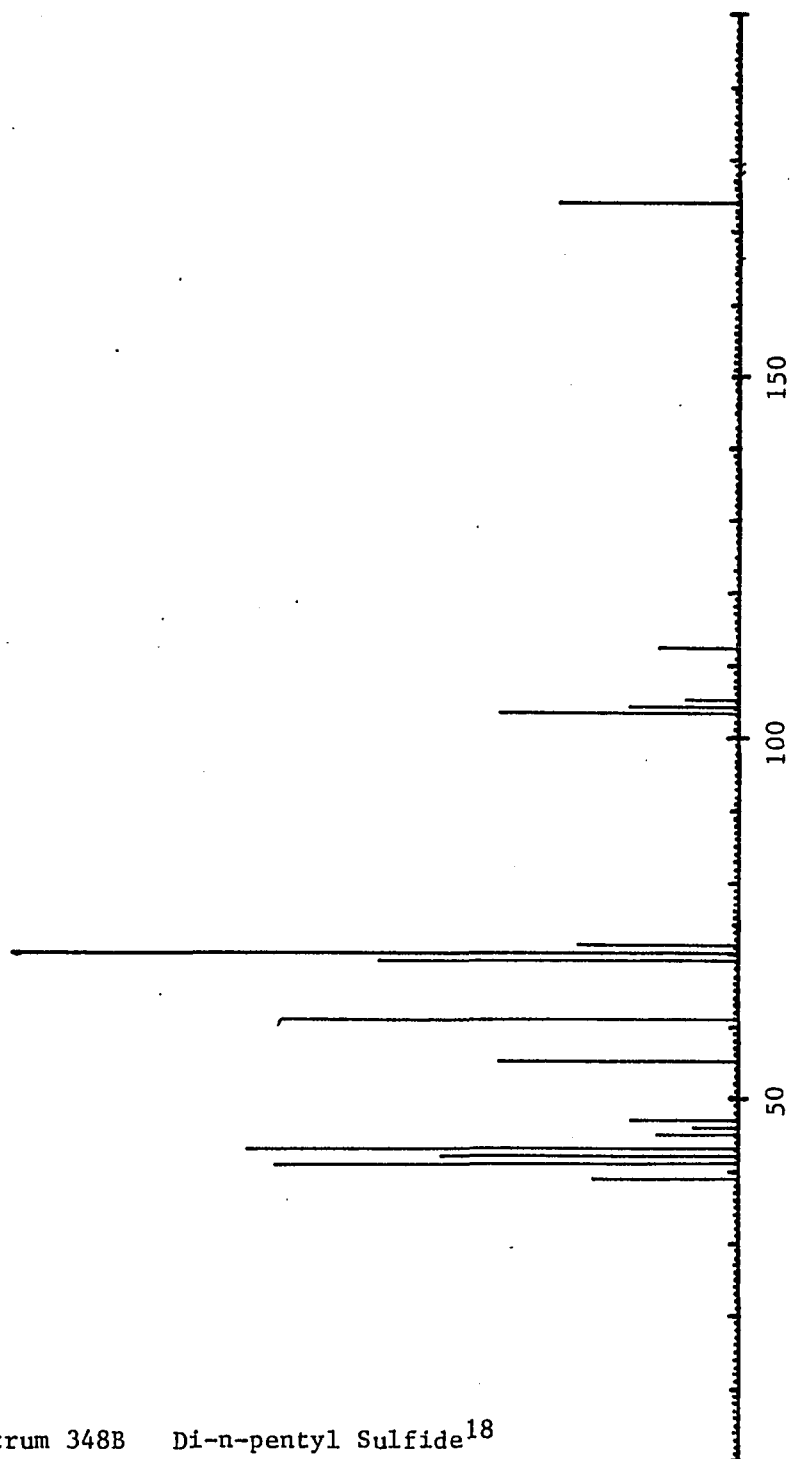
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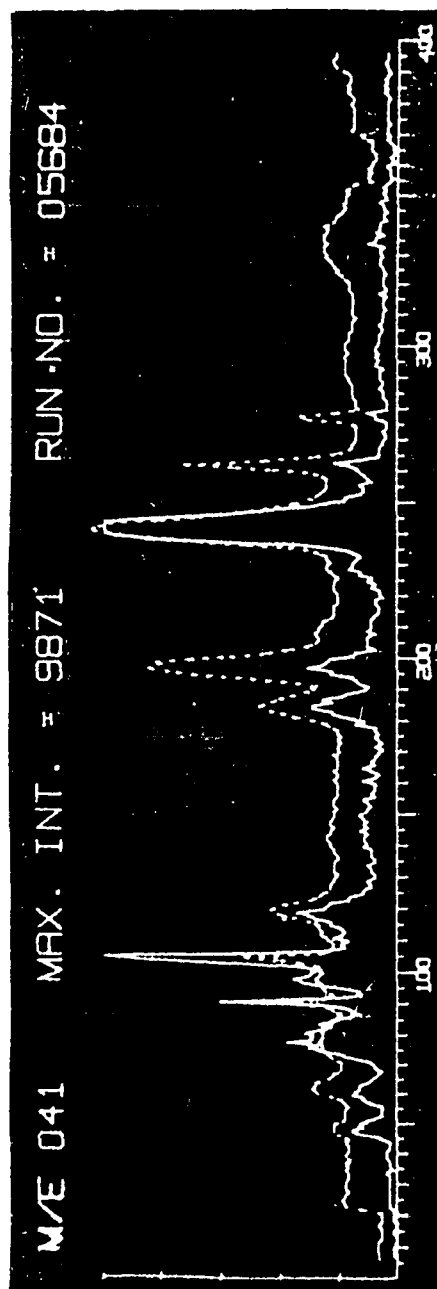
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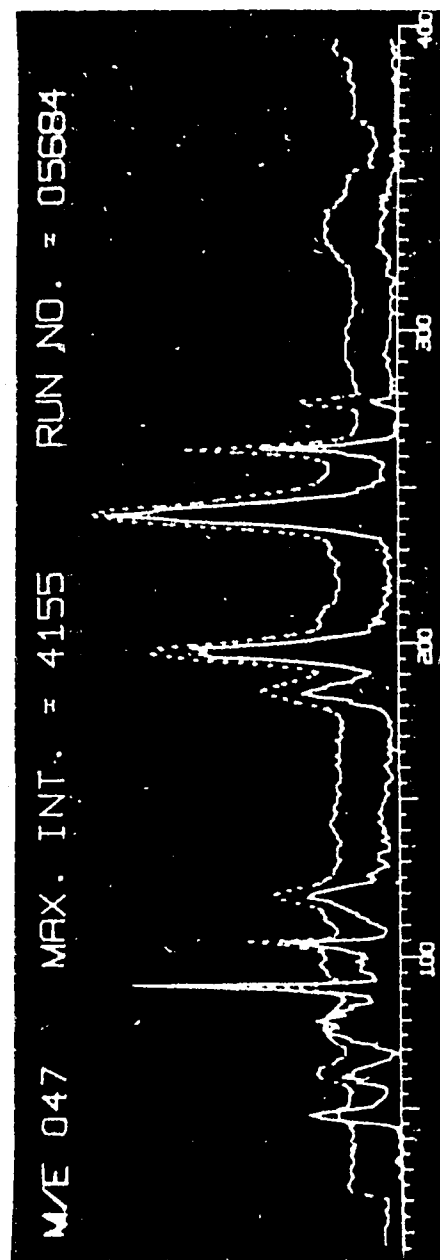
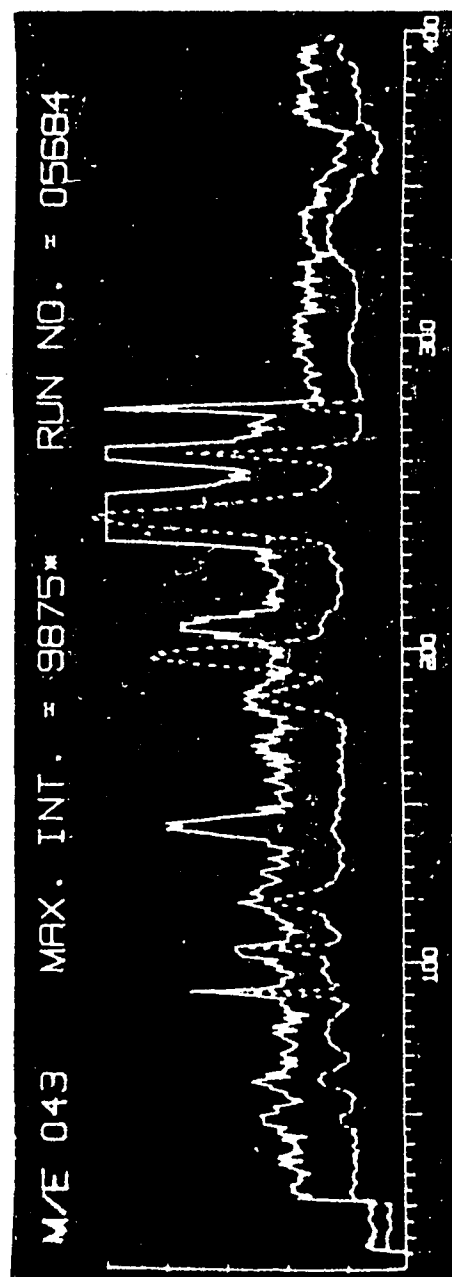
Mass Spectrum 348A Diisopentyl Sulfide¹⁸



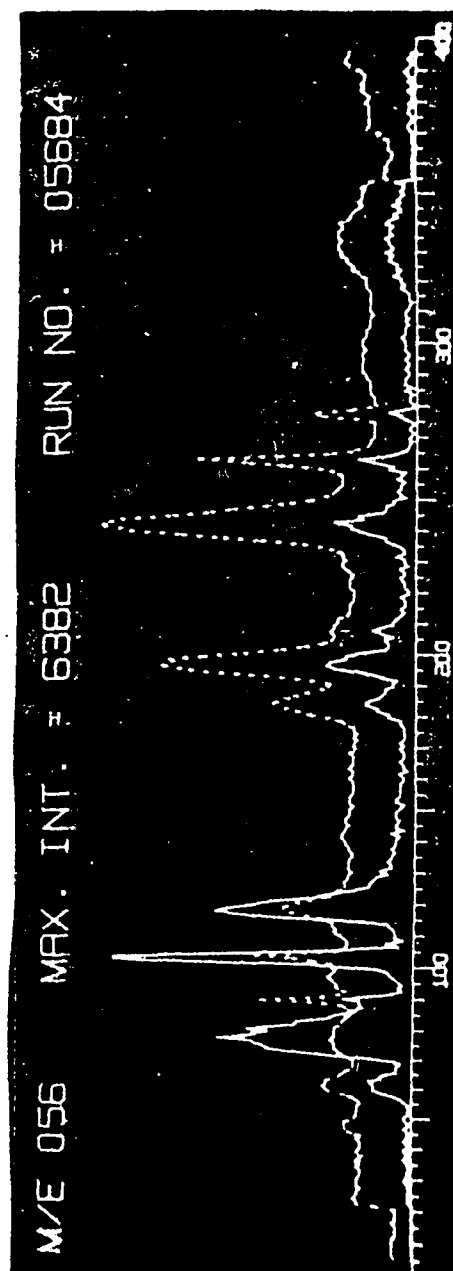
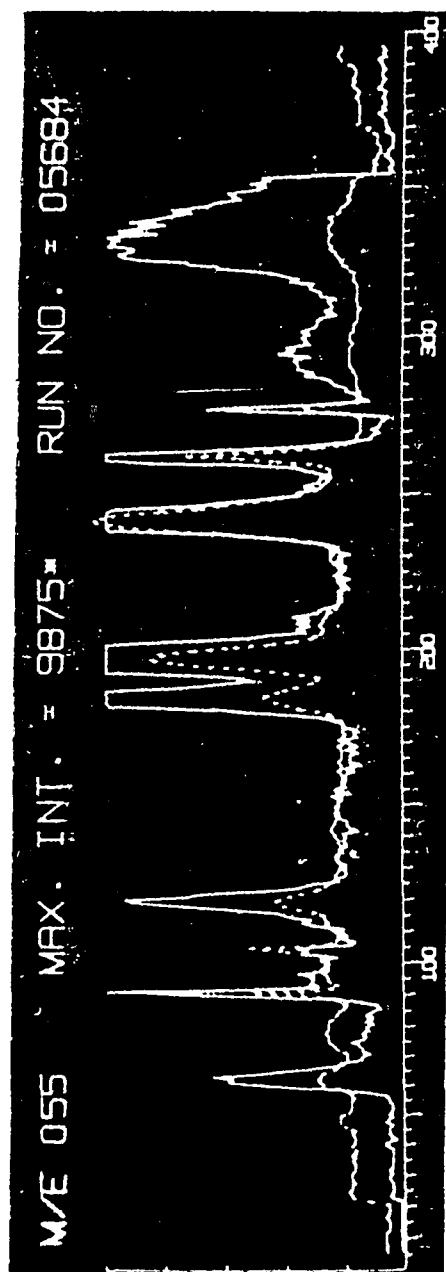
Mass Spectrum 348B Di-n-pentyl Sulfide¹⁸



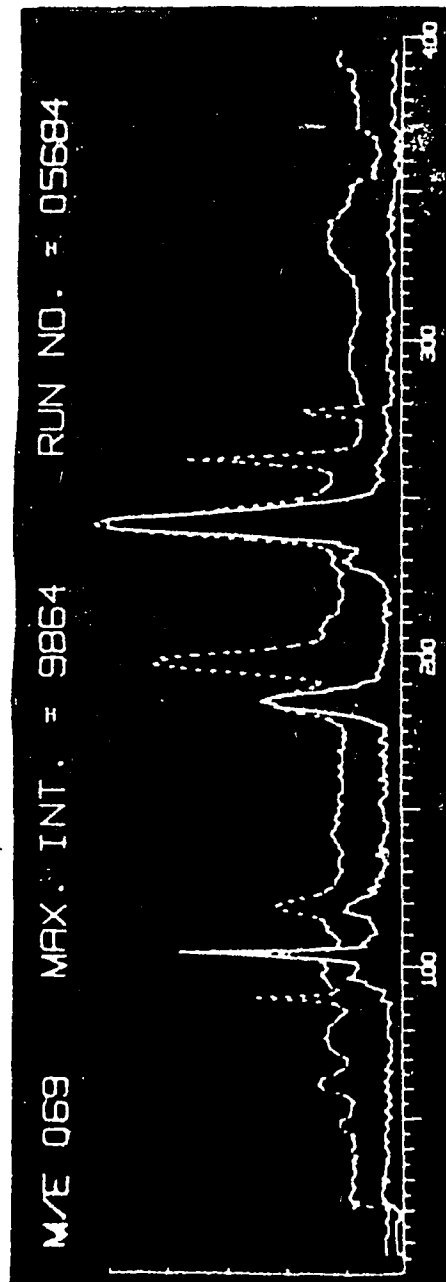
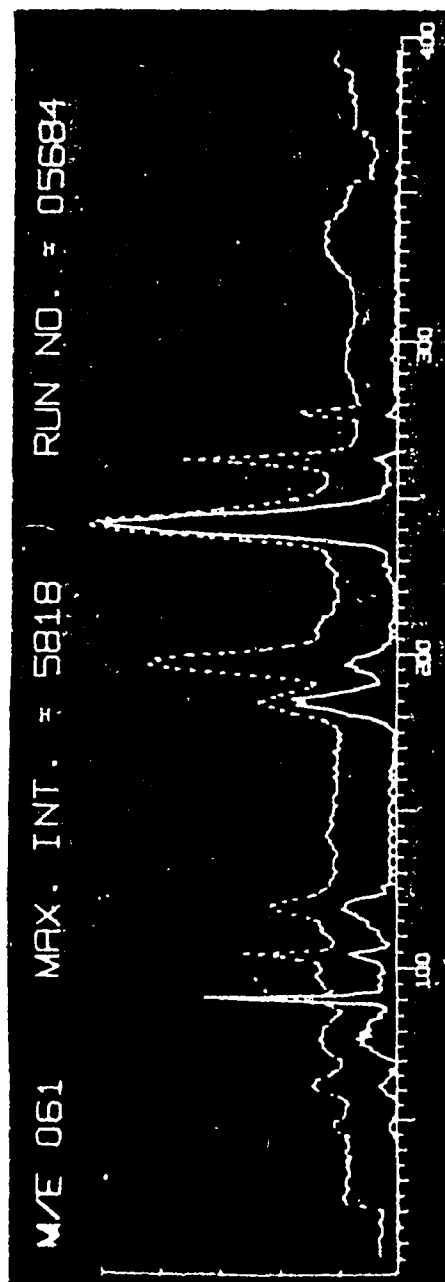
Ionization Intensity Plot of m/e 41.



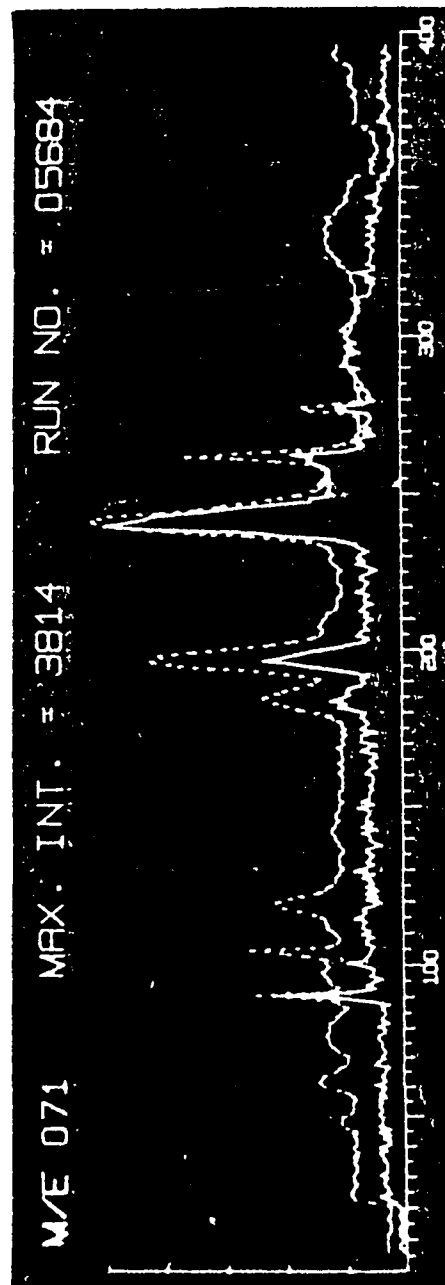
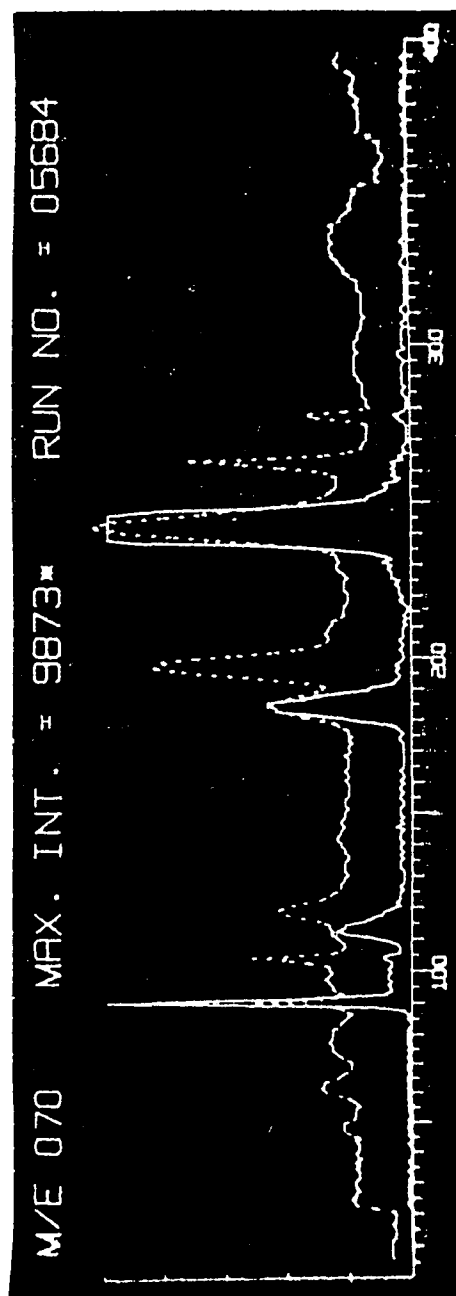
Ionization Intensity Plots of m/e 43 and m/e 47.



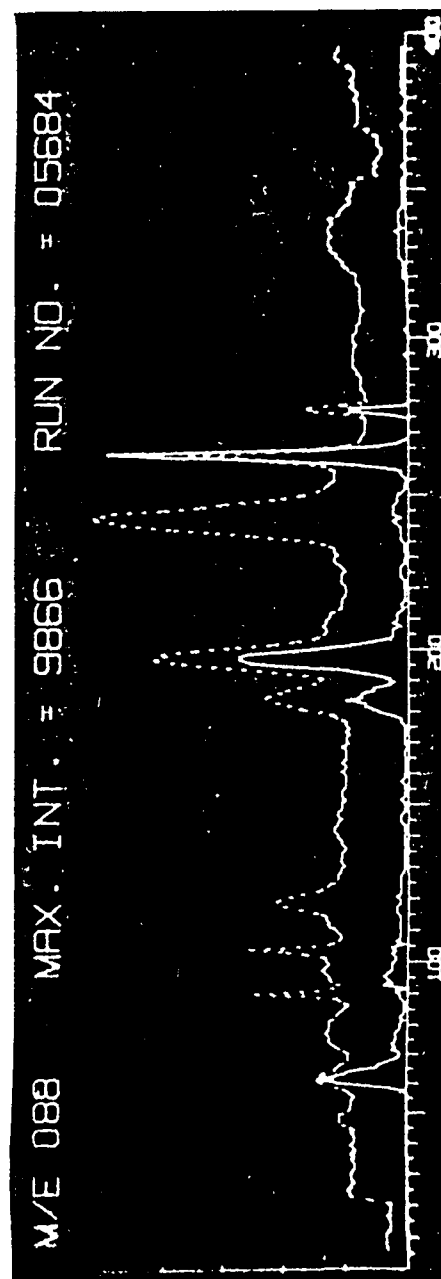
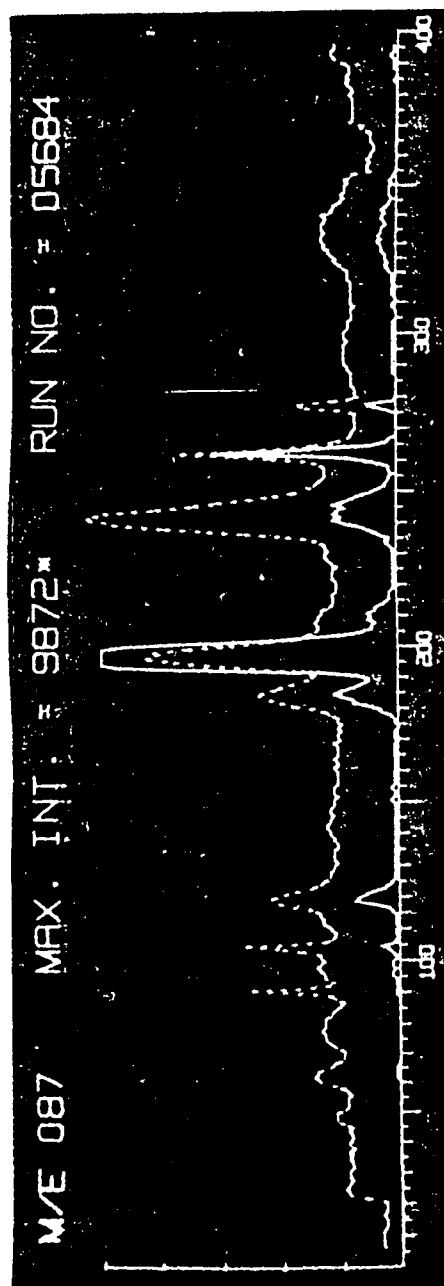
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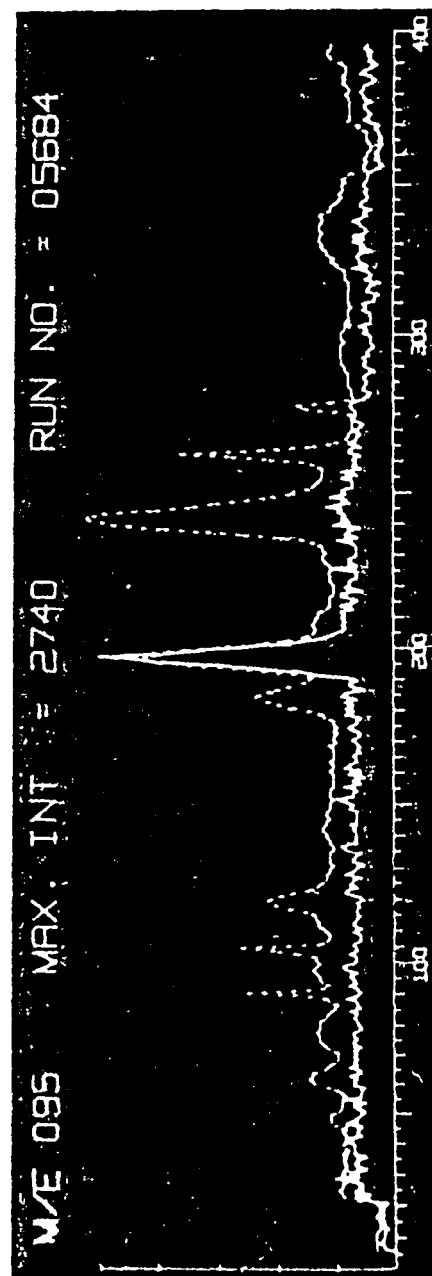
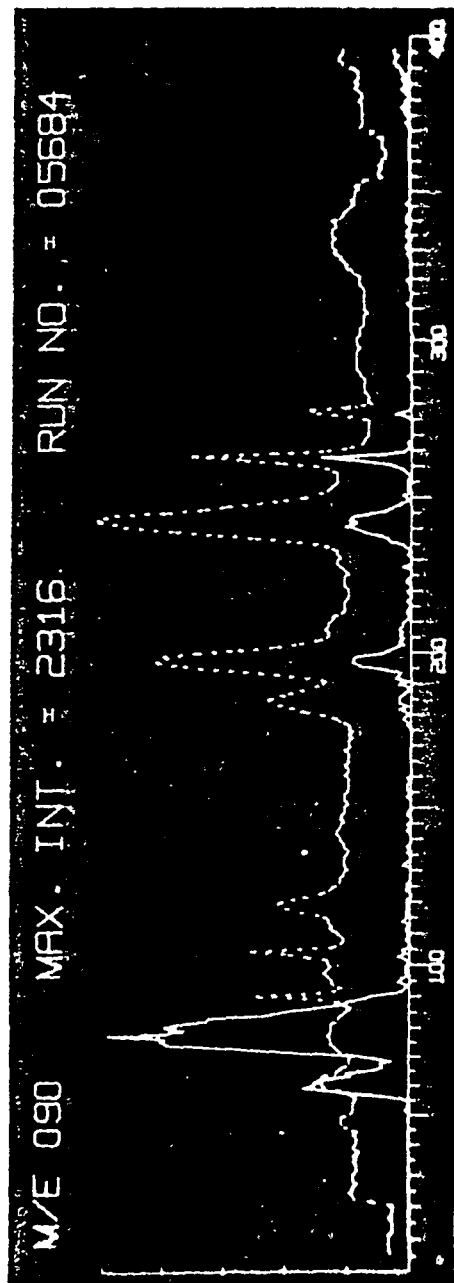
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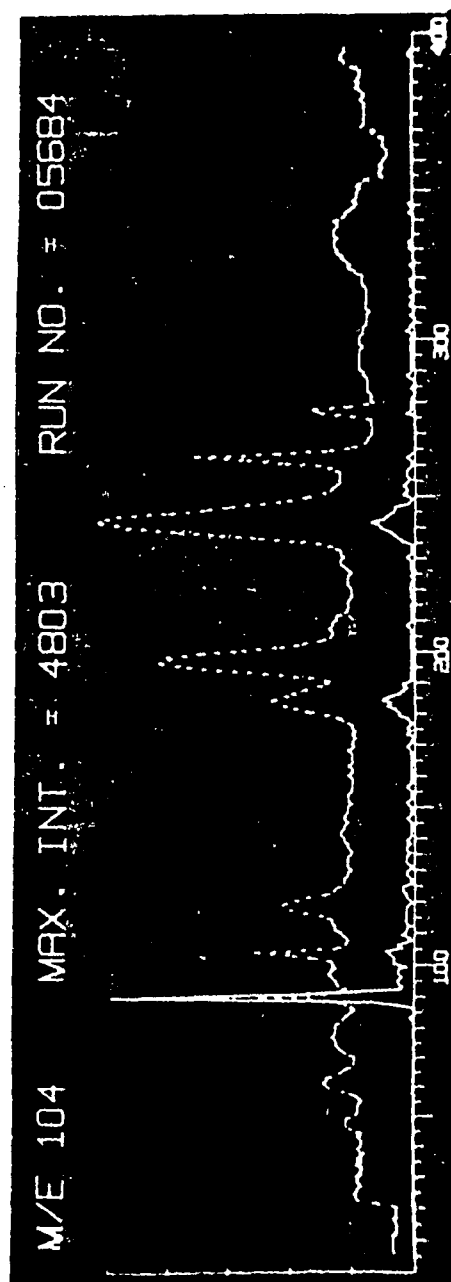
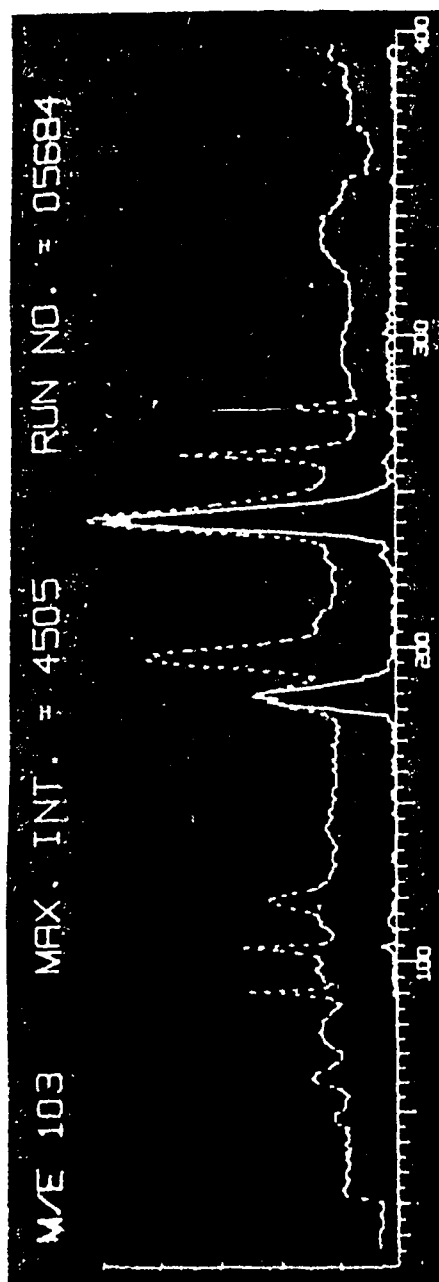
Ionization Intensity Plots of m/e 70 and m/e 71.



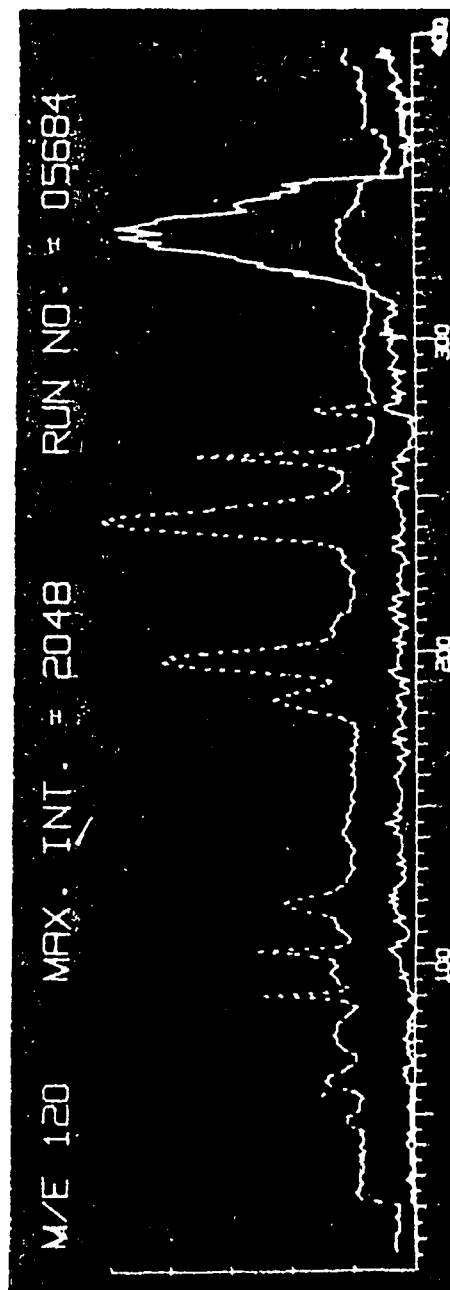
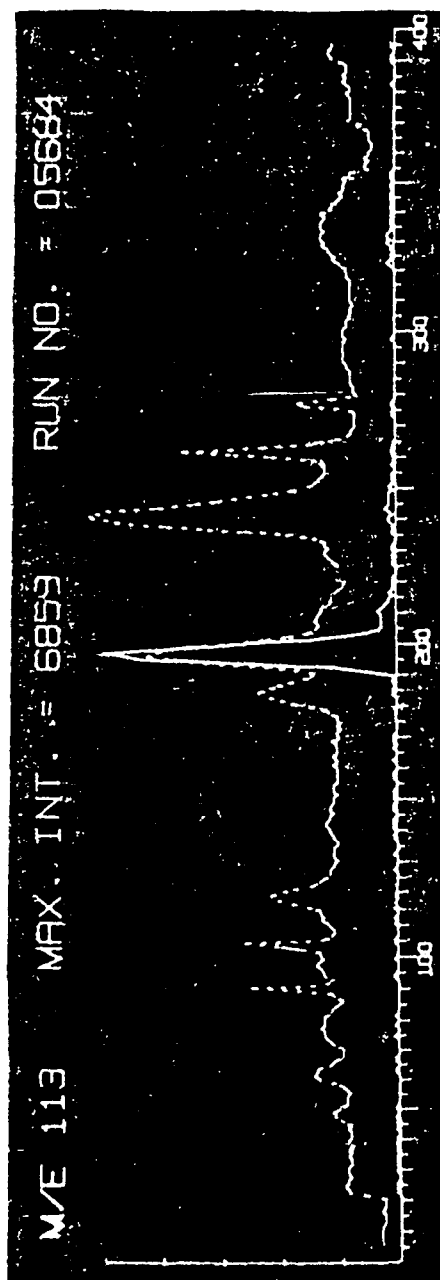
Ionization Intensity Plots of m/e 87 and m/e 88.



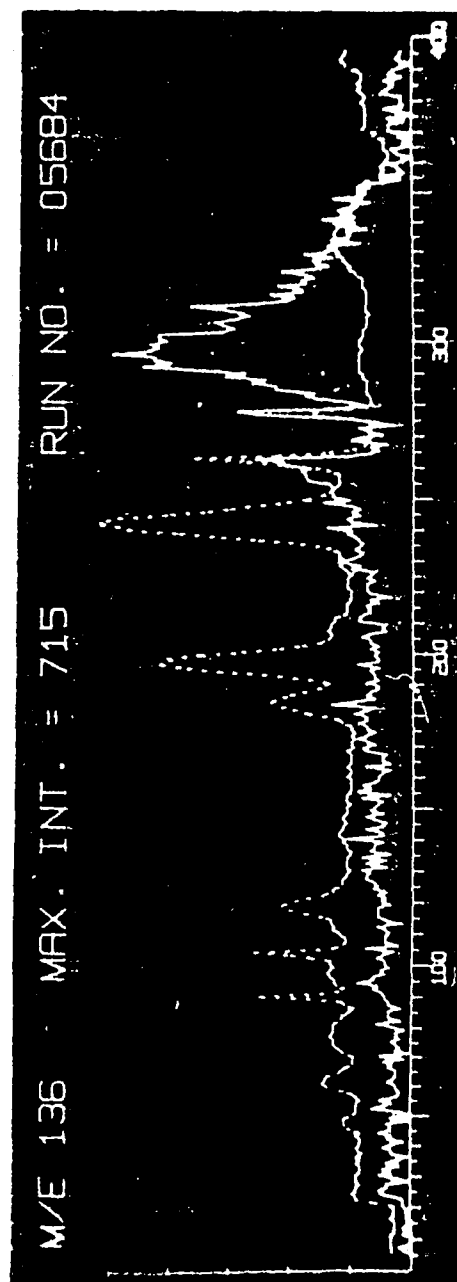
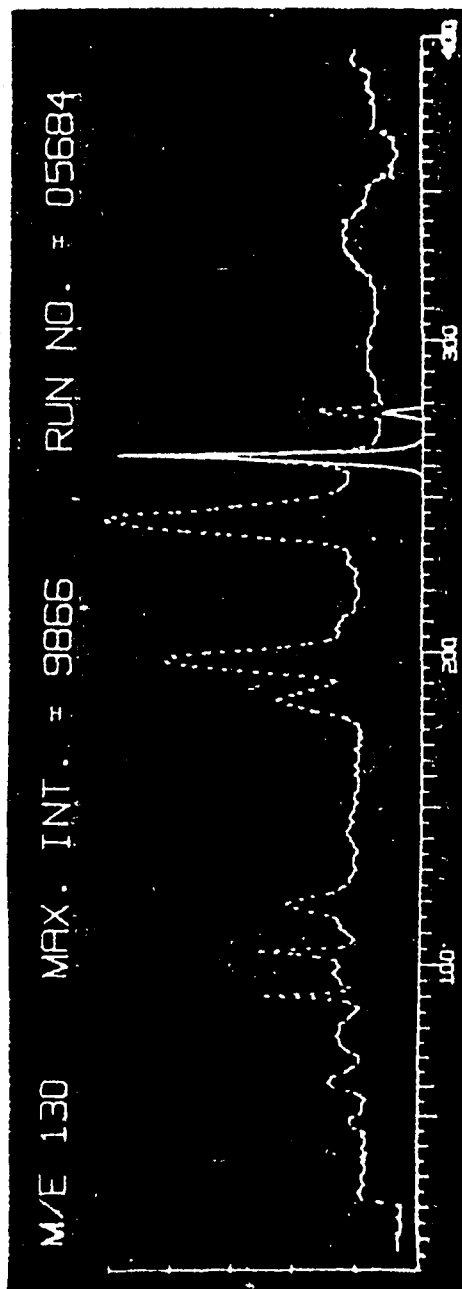
Ionization Intensity Plots of m/e 90 and m/e 95



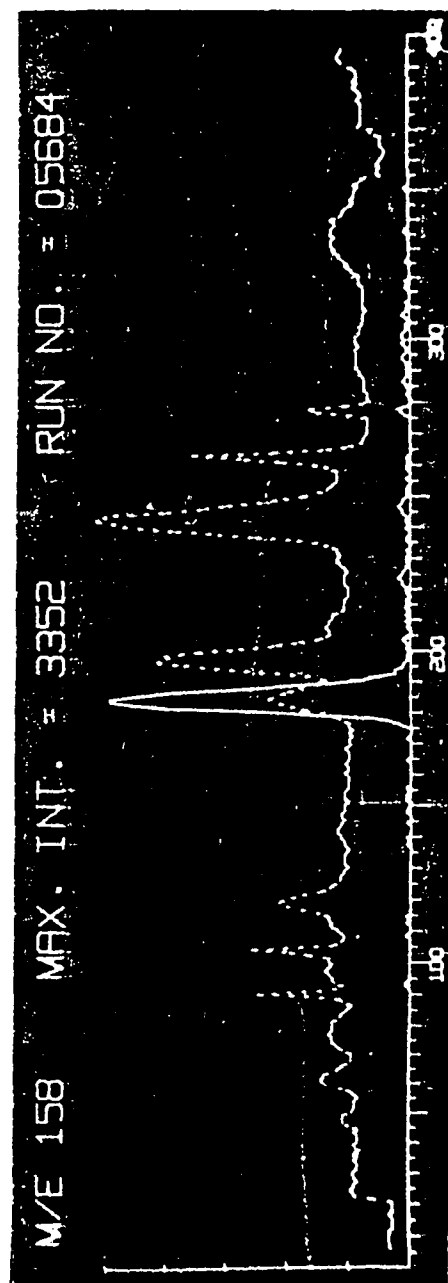
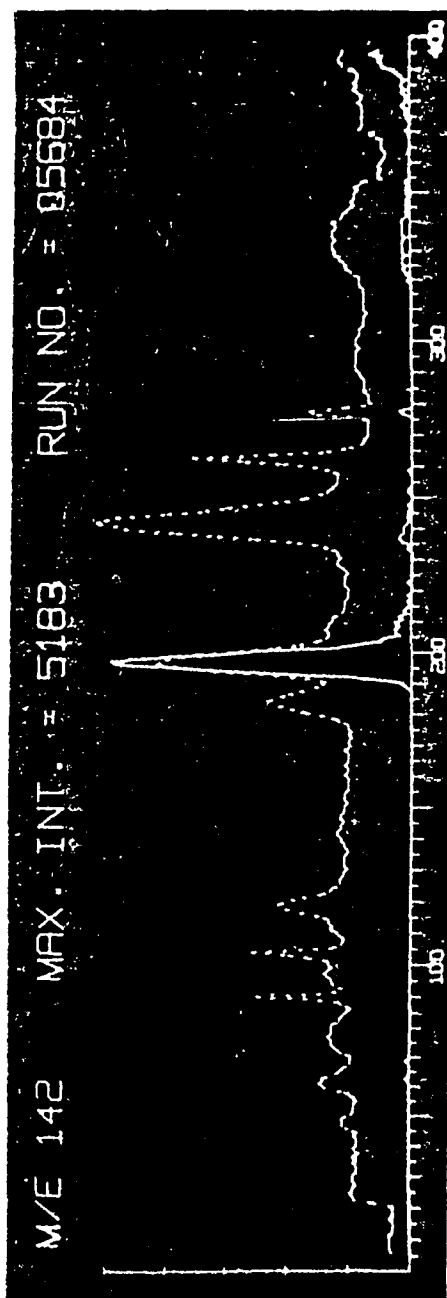
Ionization Intensity Plots of m/e 103 and m/e 104.



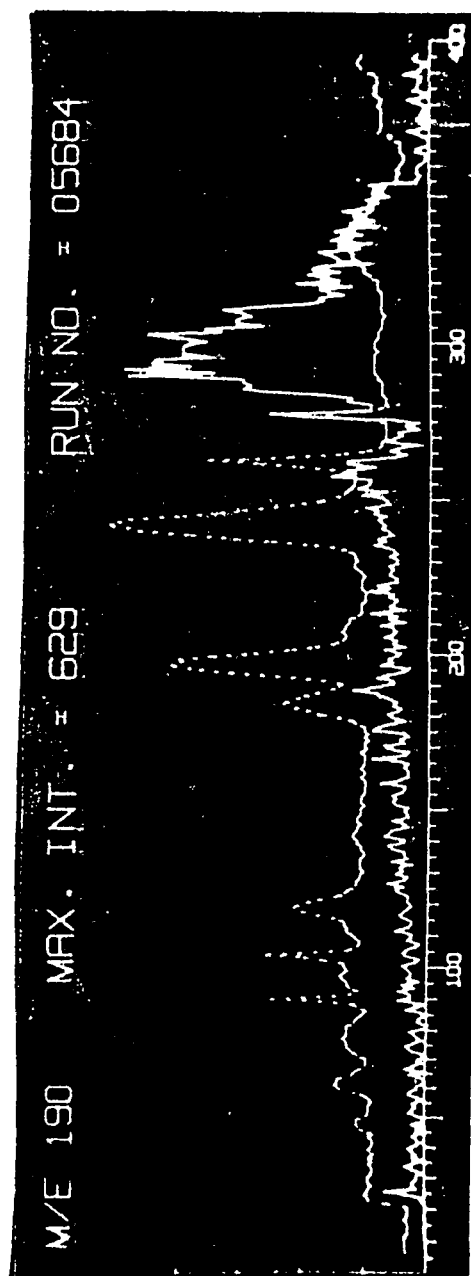
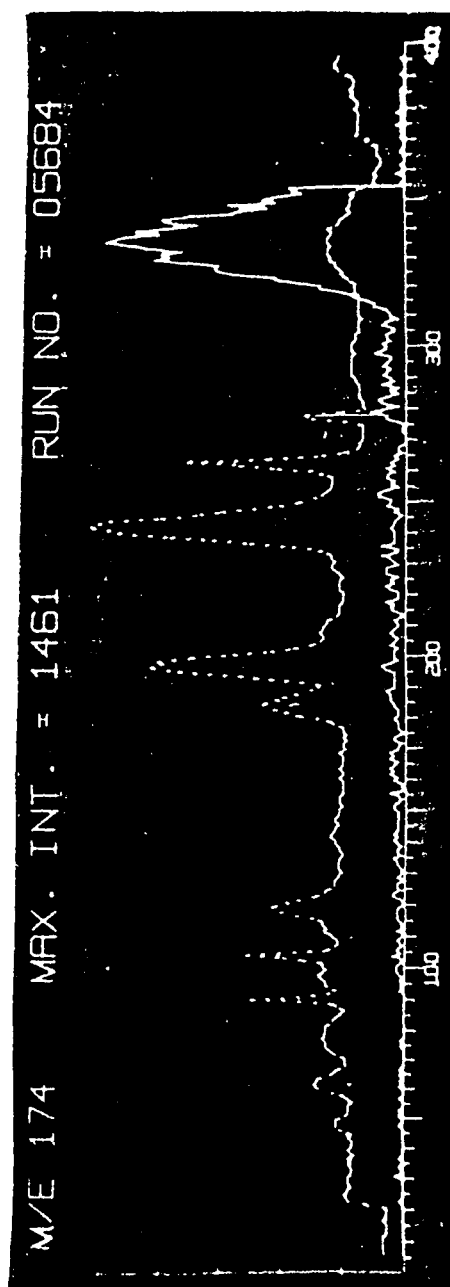
Ionization Intensity Plots of m/e 113 and m/e 120.



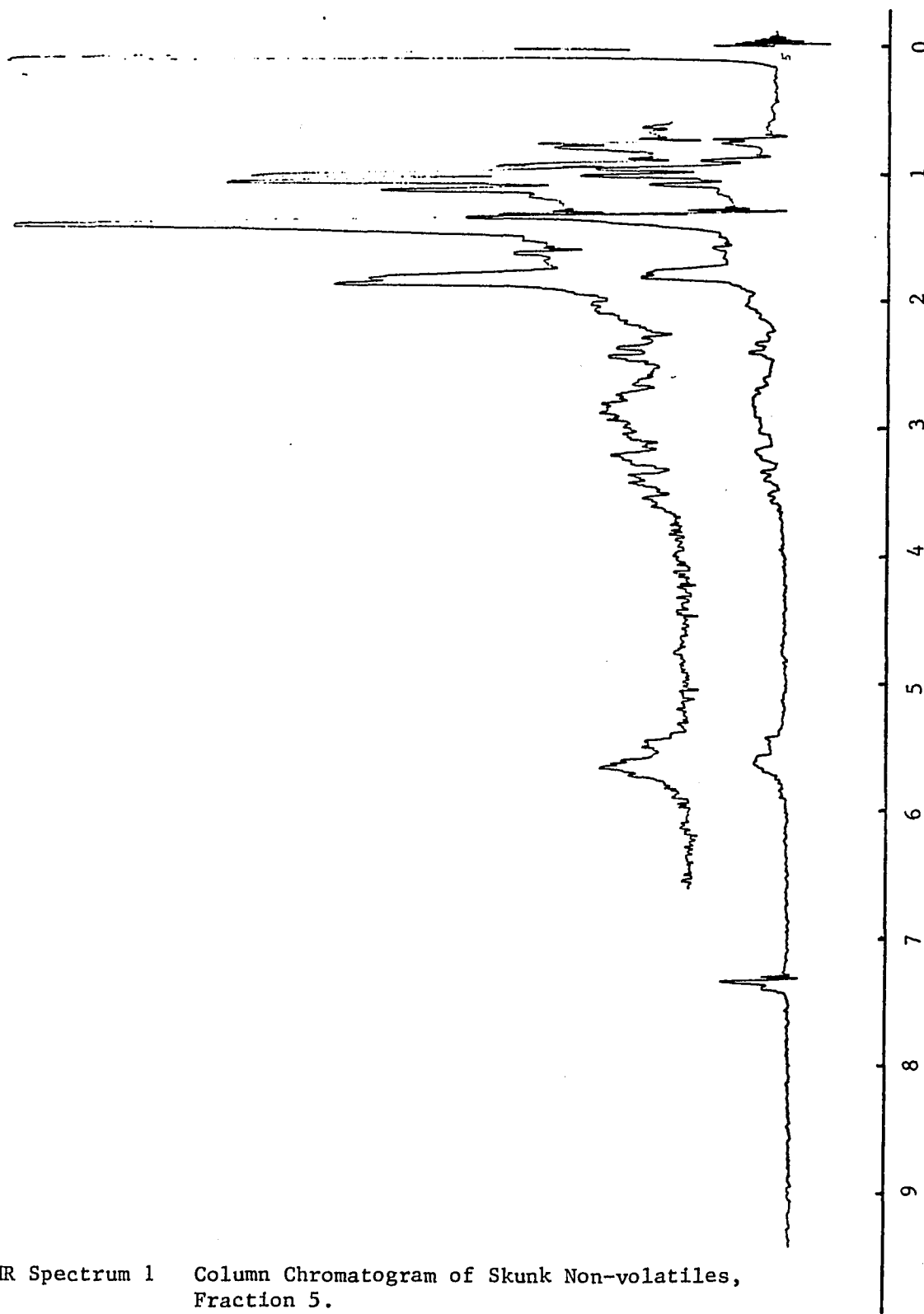
Ionization Intensity Plots of m/e 130 and m/e 136.



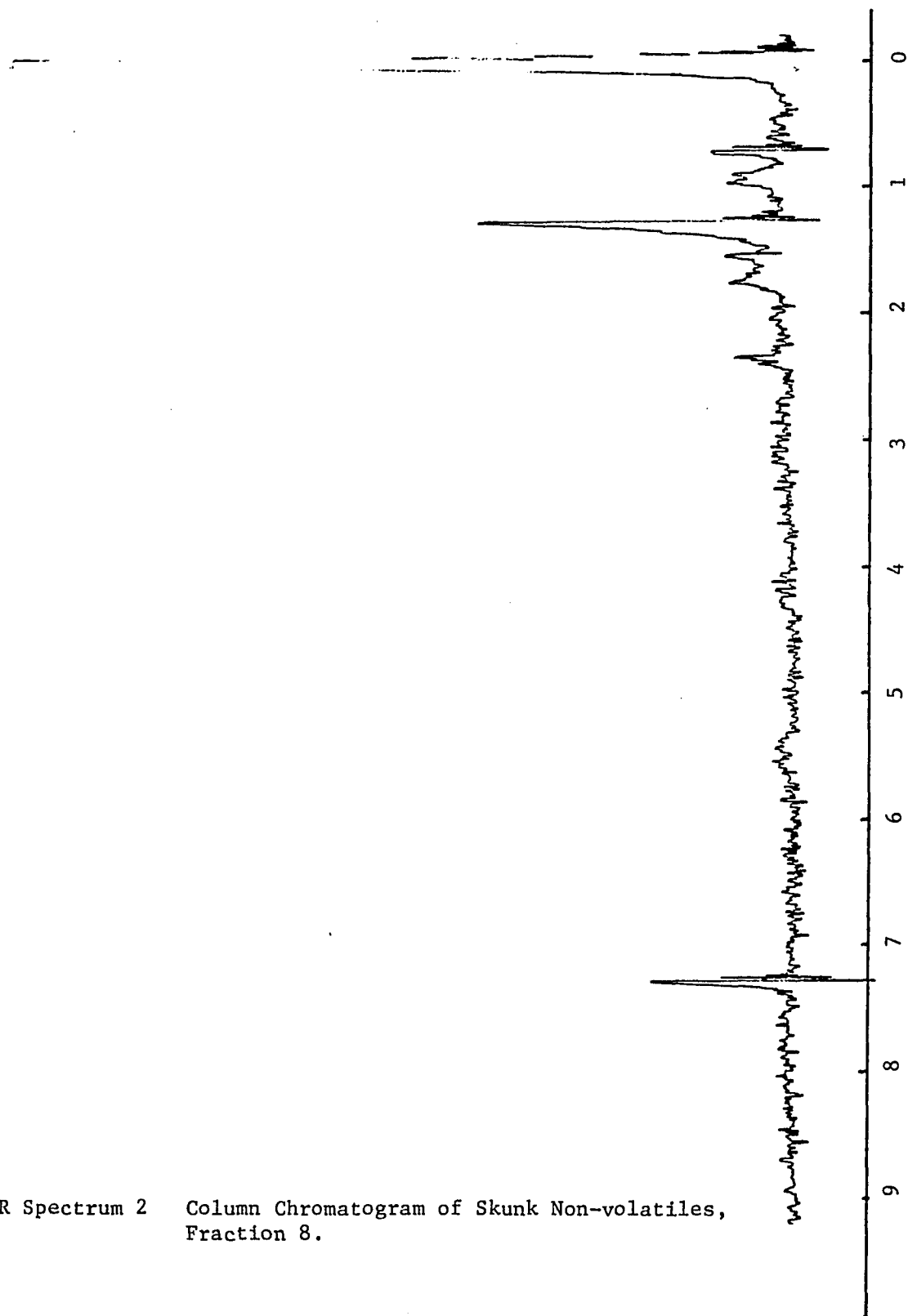
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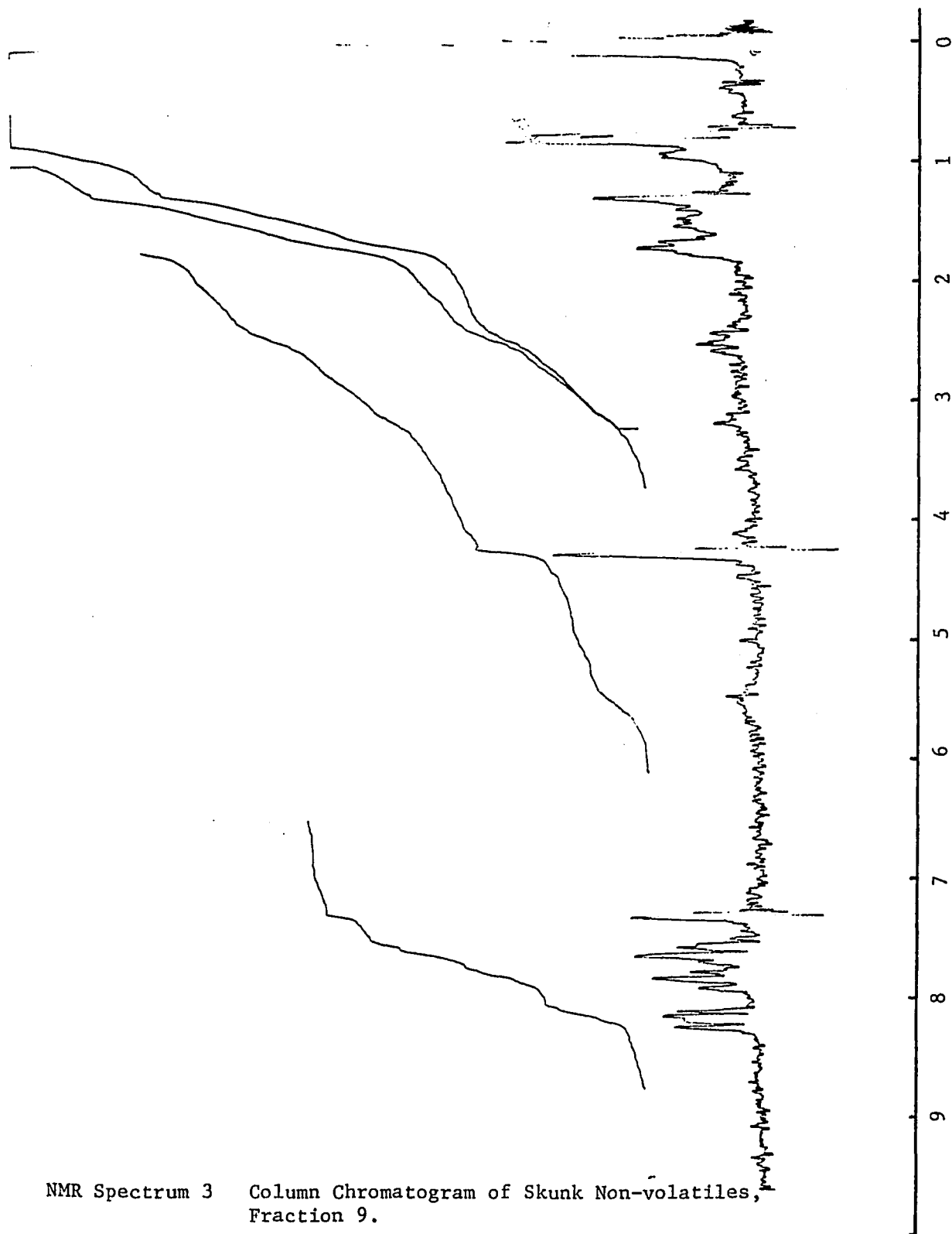
Ionization Intensity Plots of m/e 174 and m/e 190.

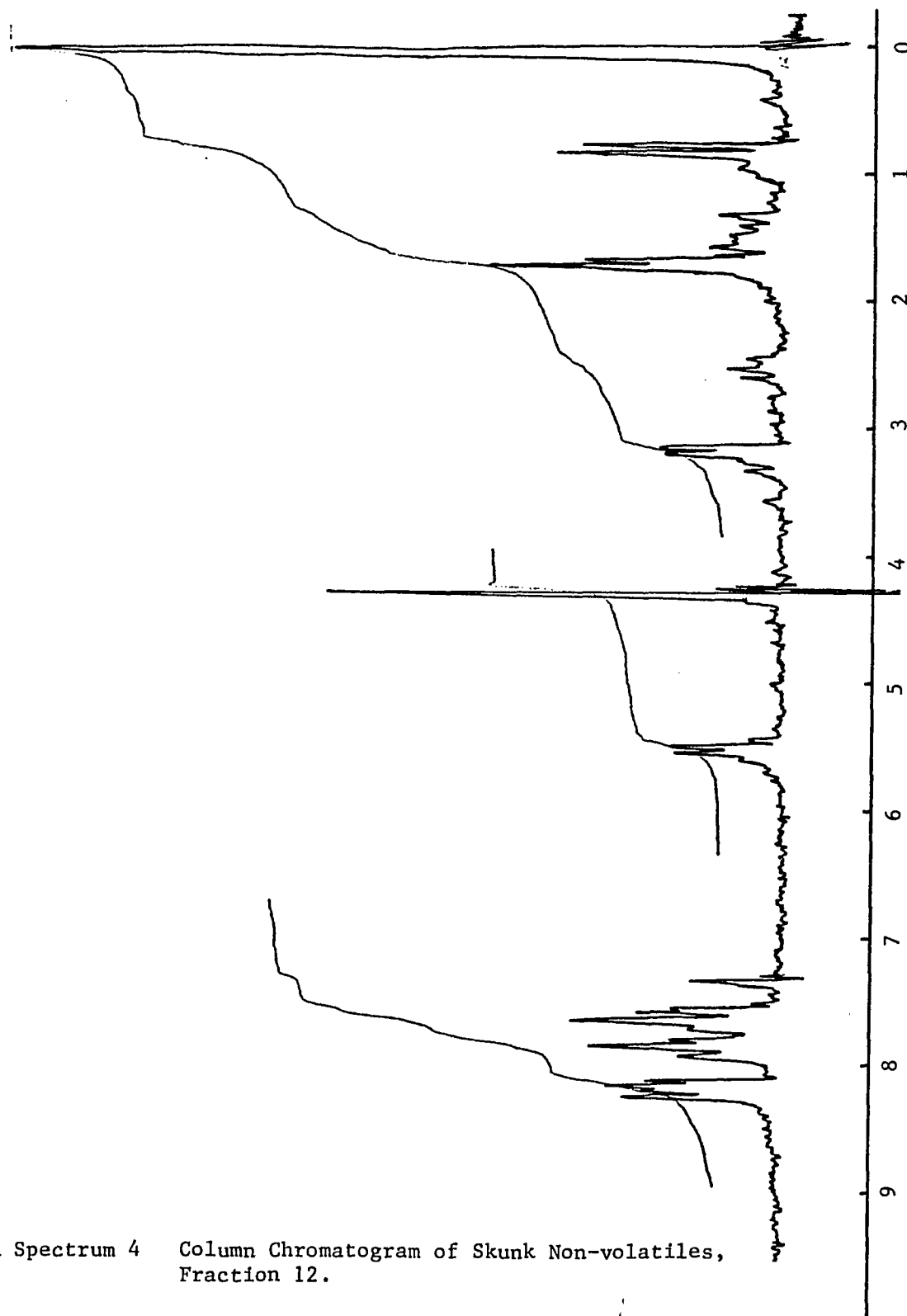


NMR Spectrum 1 Column Chromatogram of Skunk Non-volatiles,
Fraction 5.

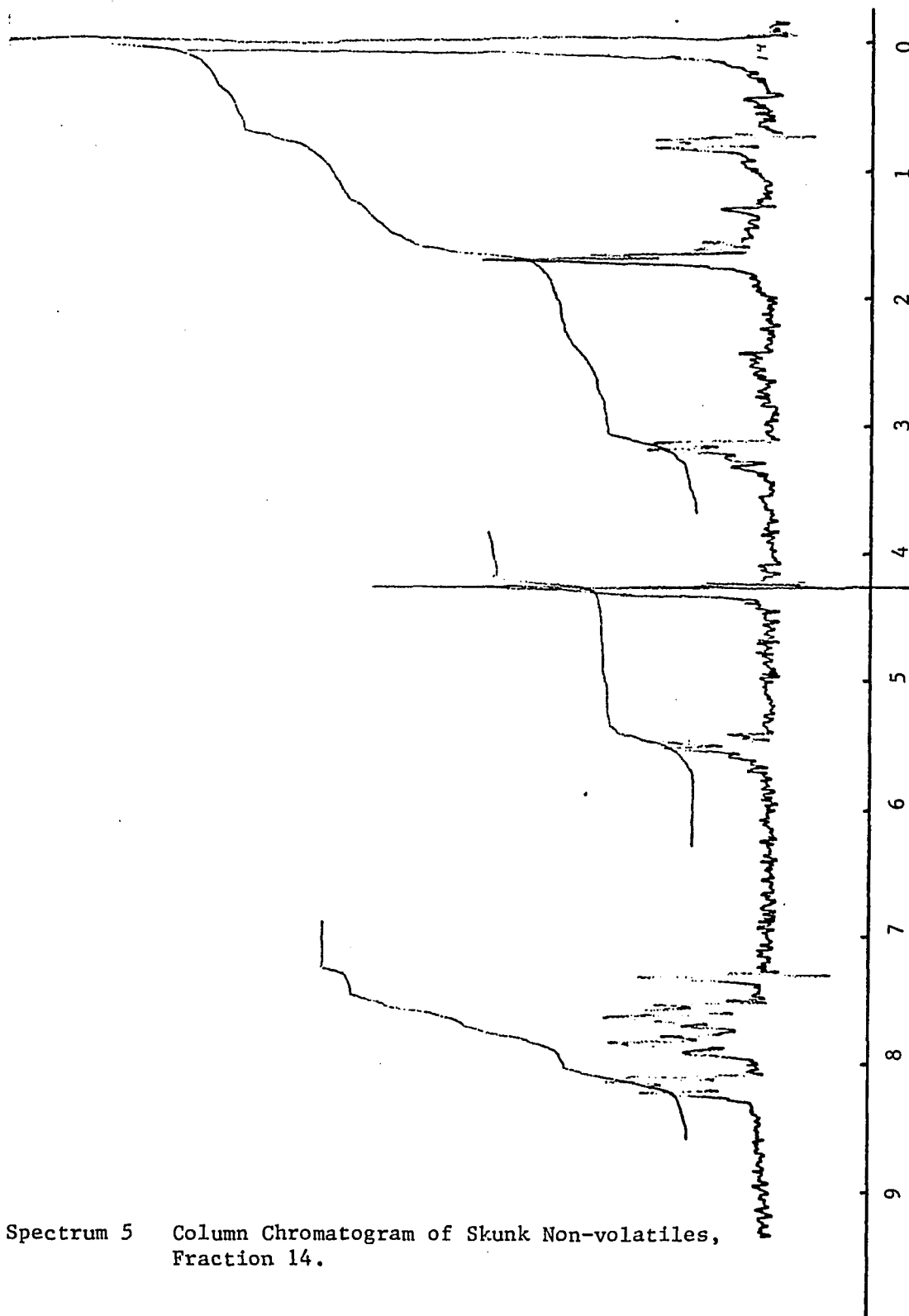


NMR Spectrum 2 Column Chromatogram of Skunk Non-volatiles,
Fraction 8.

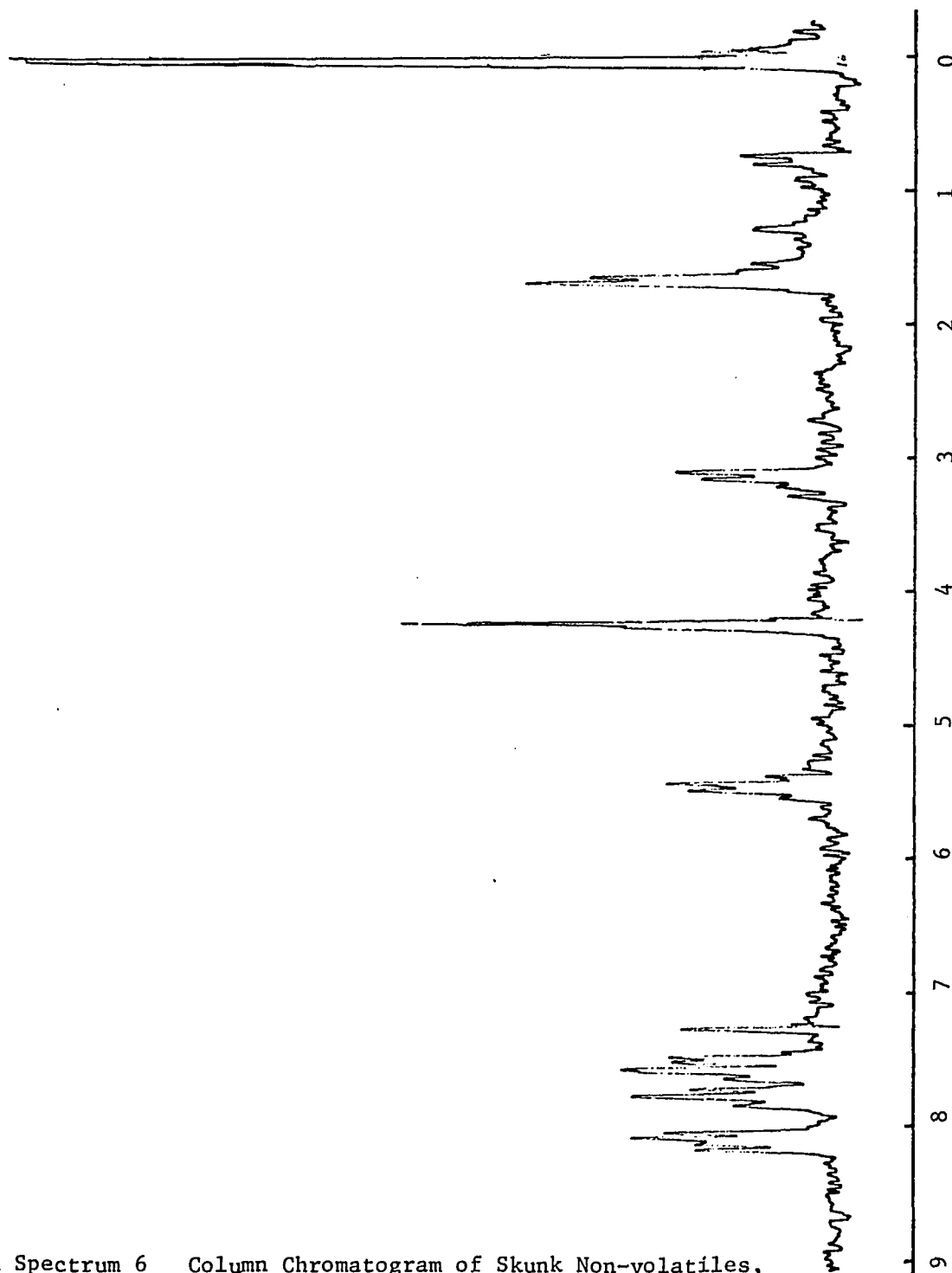




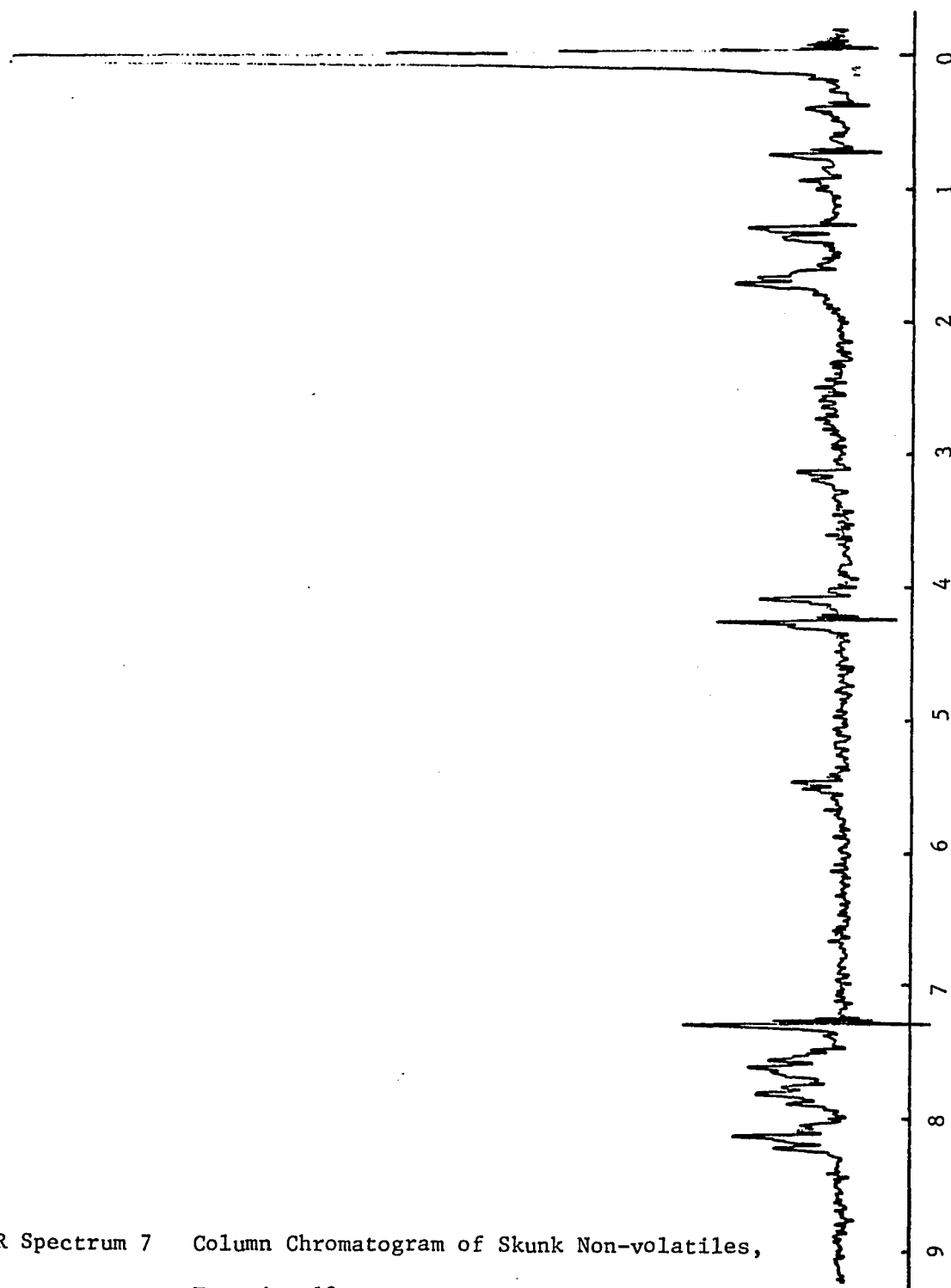
NMR Spectrum 4 Column Chromatogram of Skunk Non-volatiles,
Fraction 12.



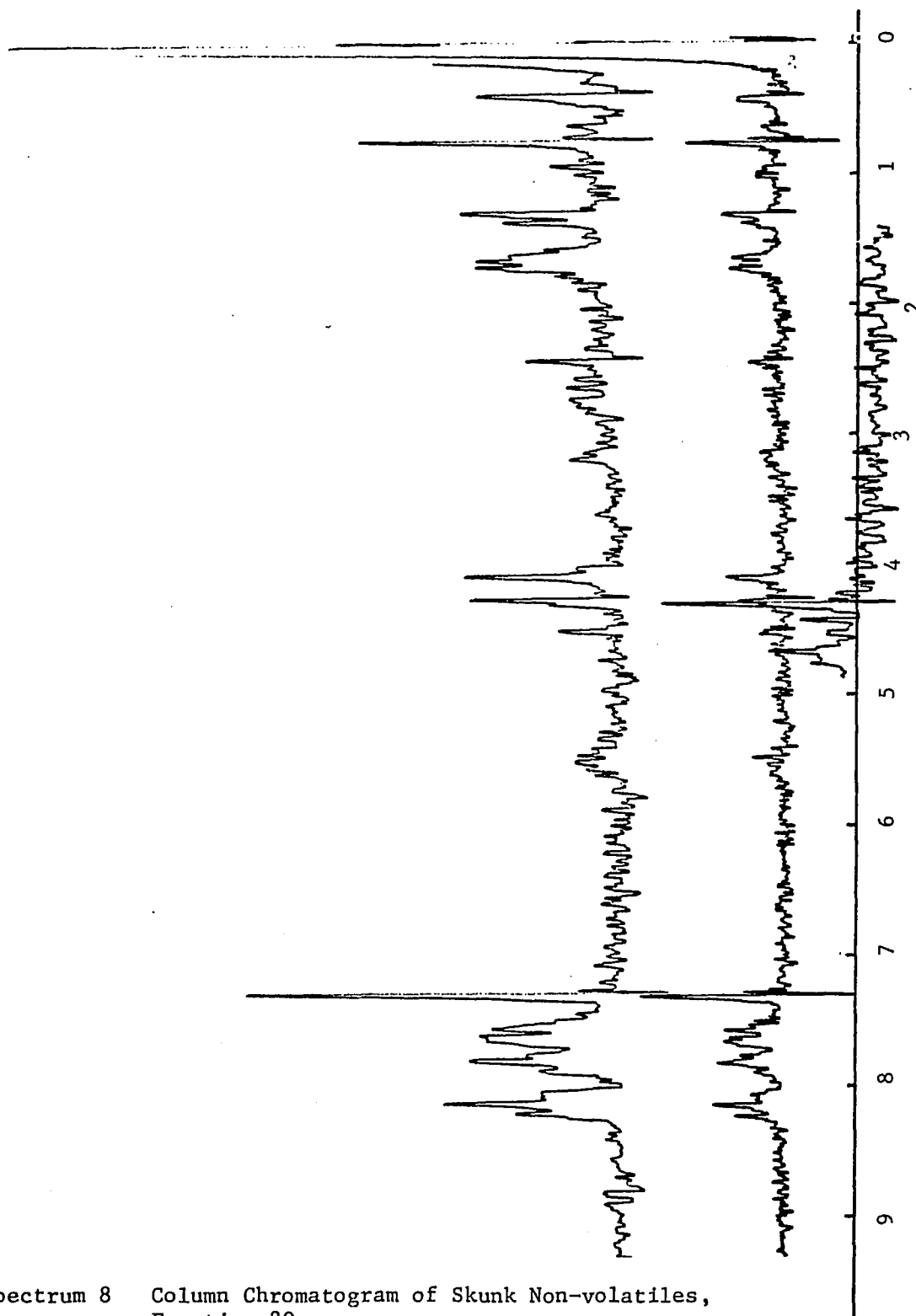
NMR Spectrum 5 Column Chromatogram of Skunk Non-volatiles,
Fraction 14.



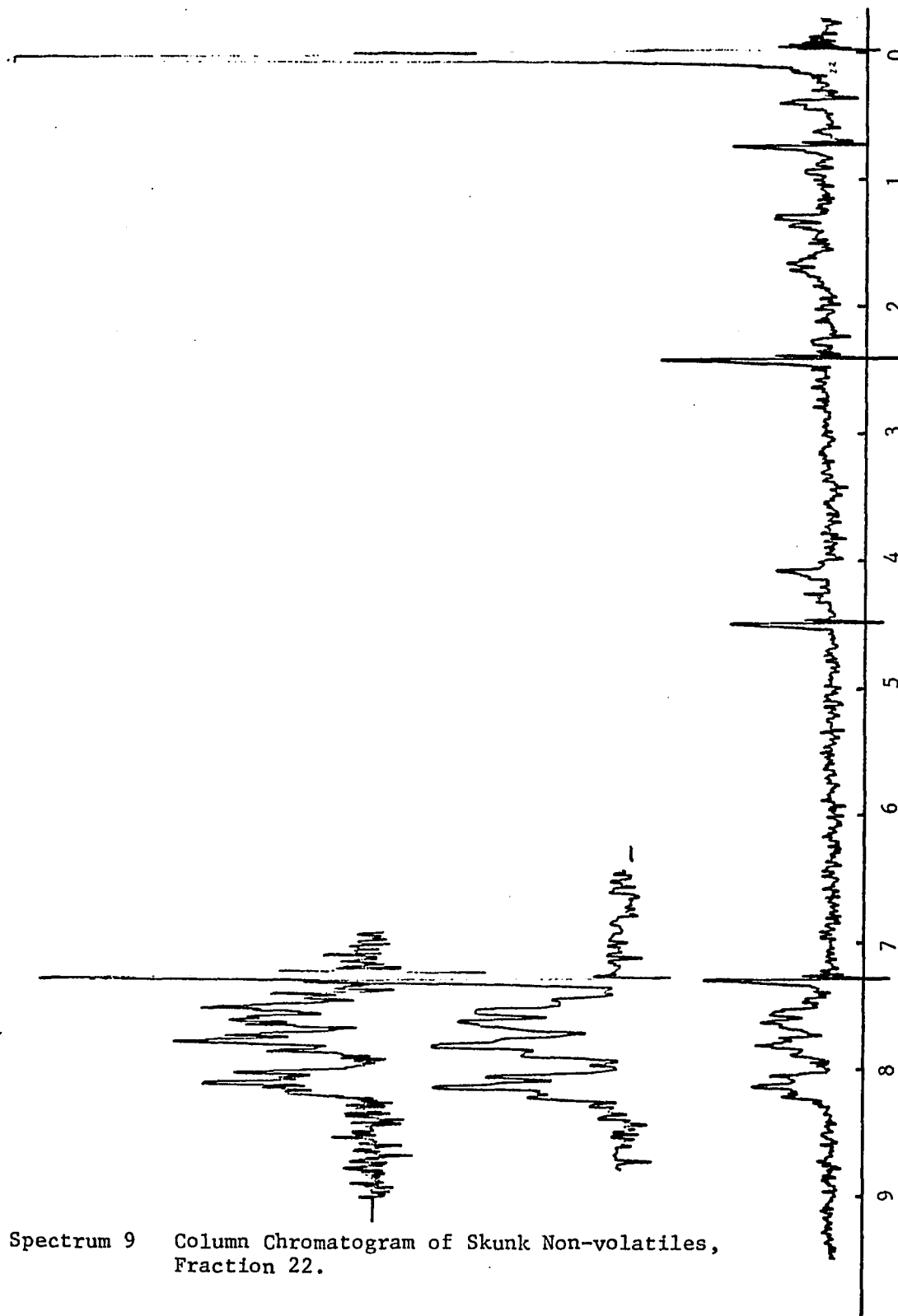
NMR Spectrum 6 Column Chromatogram of Skunk Non-volatiles,
Fraction 16.



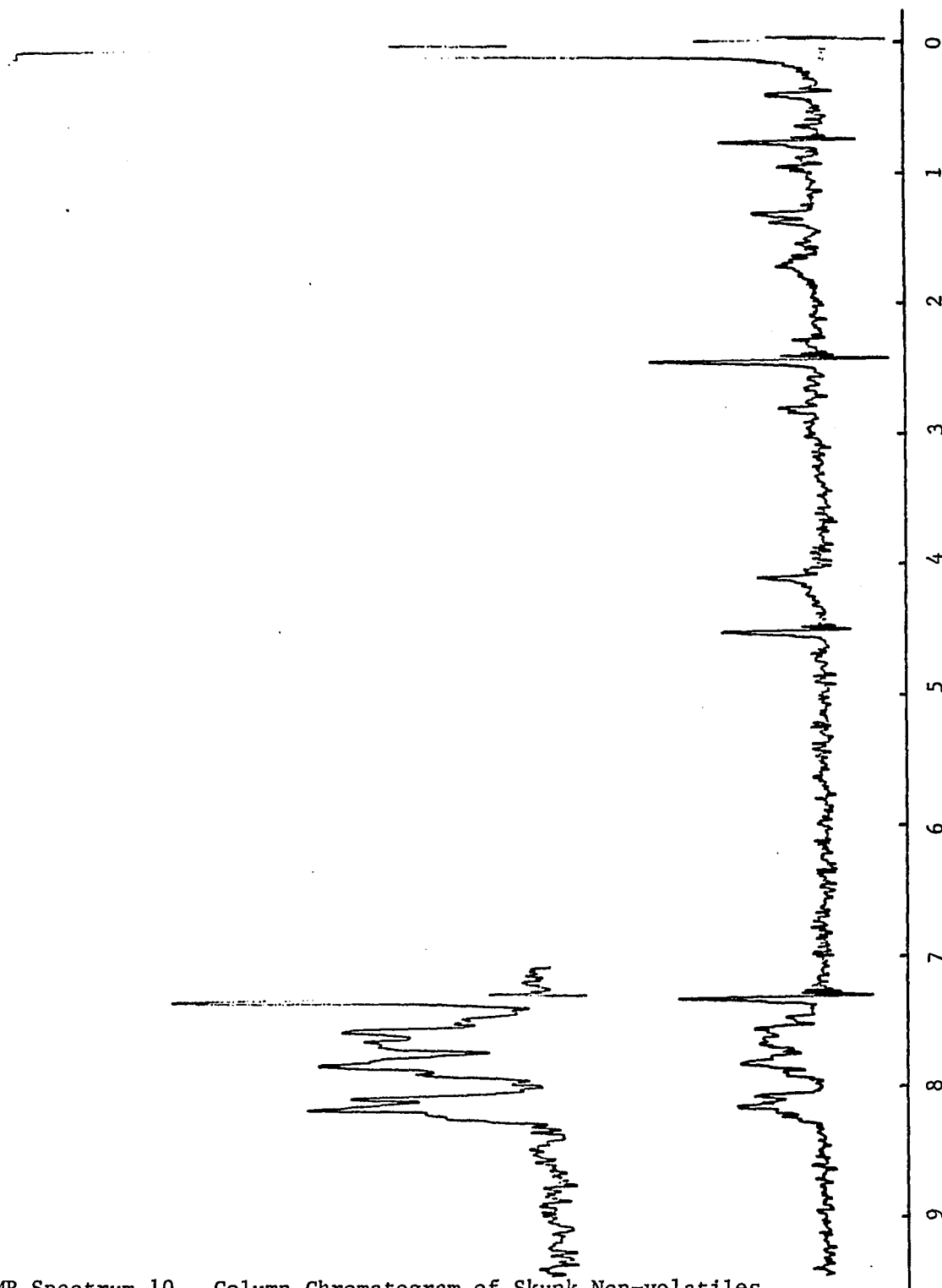
NMR Spectrum 7 Column Chromatogram of Skunk Non-volatiles,
Fraction 19



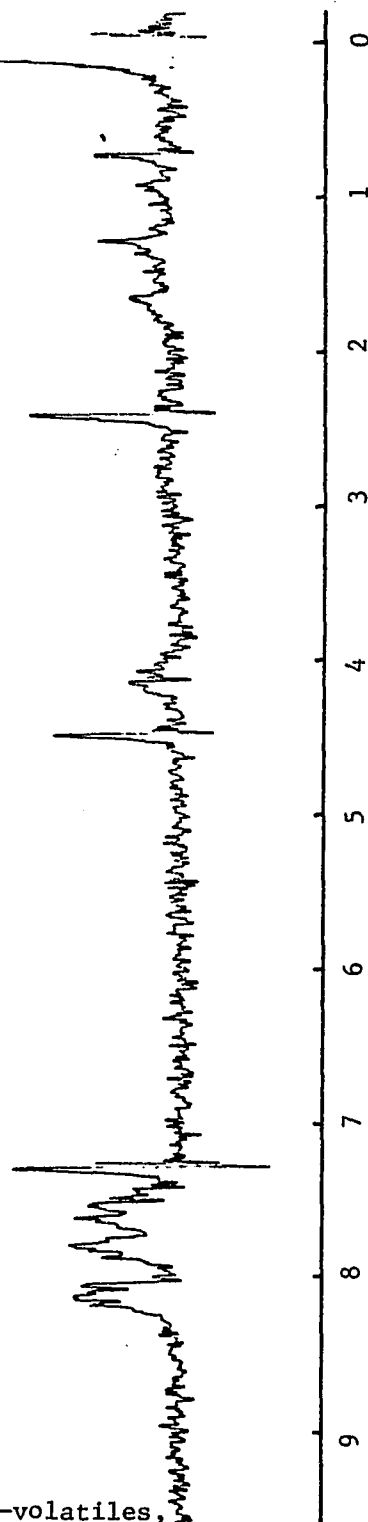
NMR Spectrum 8 Column Chromatogram of Skunk Non-volatiles,
Fraction 20



NMR Spectrum 9 Column Chromatogram of Skunk Non-volatiles, Fraction 22.

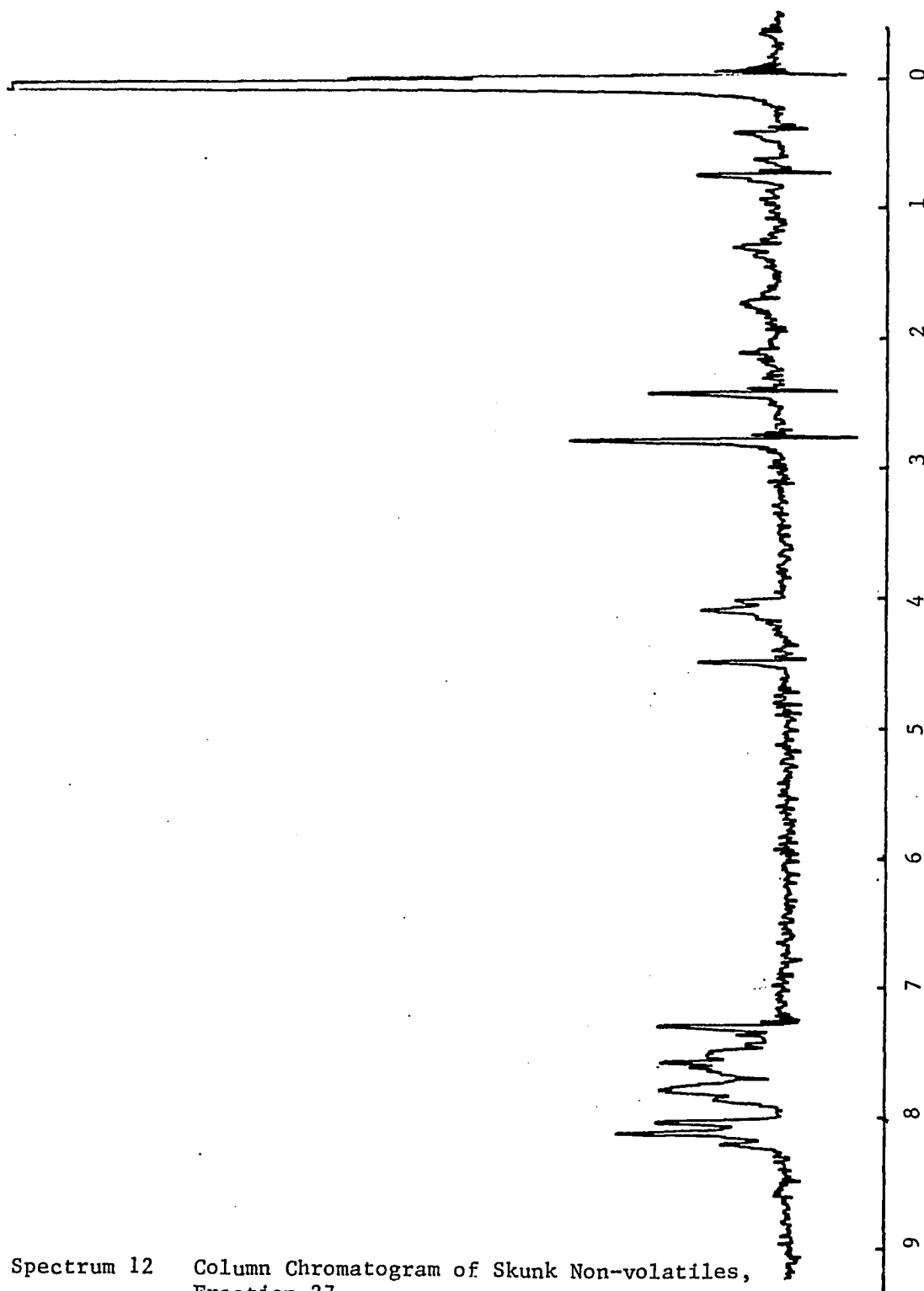


NMR Spectrum 10 Column Chromatogram of Skunk Non-volatiles,
Fraction 24.



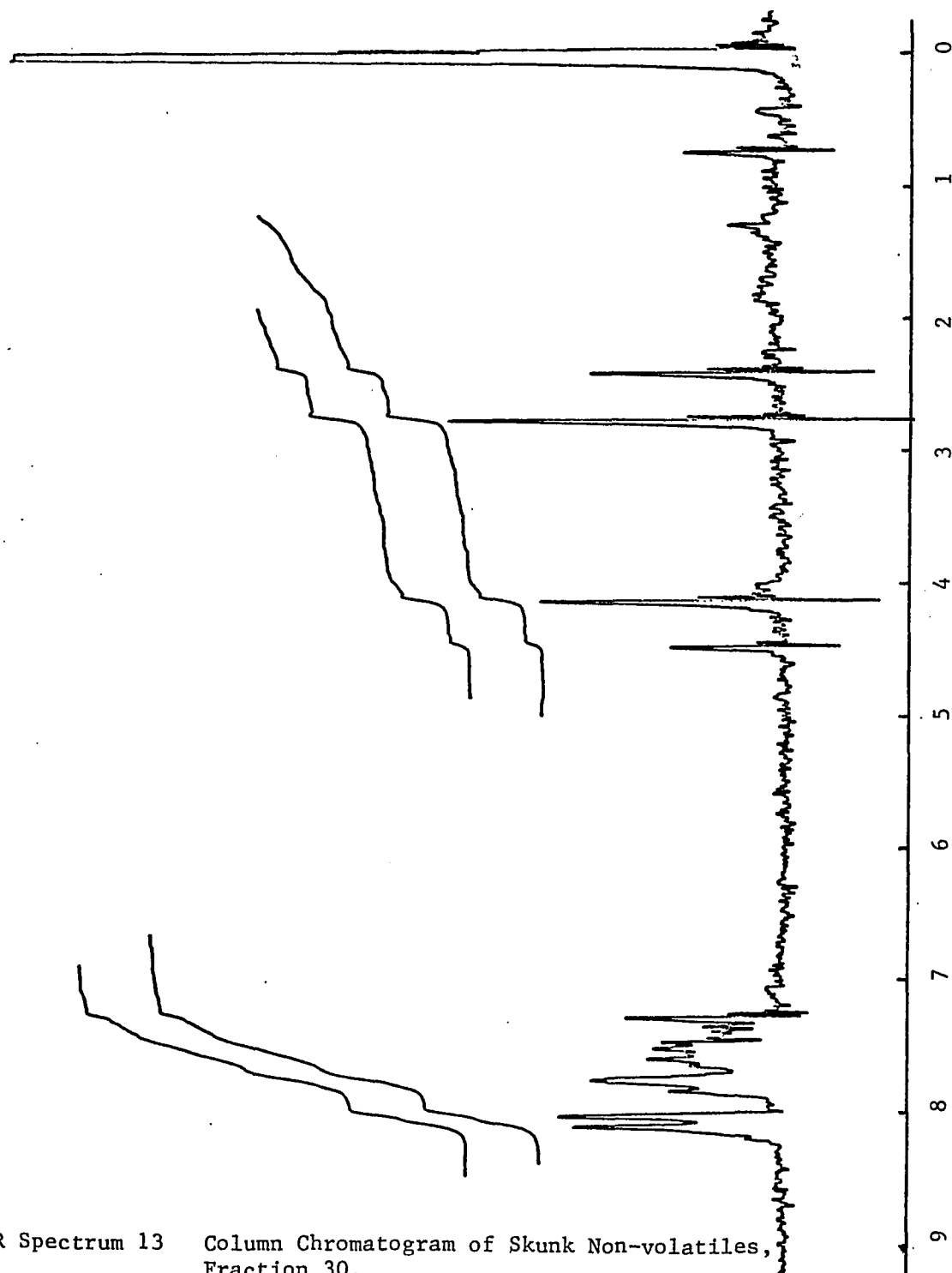
NMR Spectrum 11

Column Chromatogram of Skunk Non-volatiles,
Fraction 26.

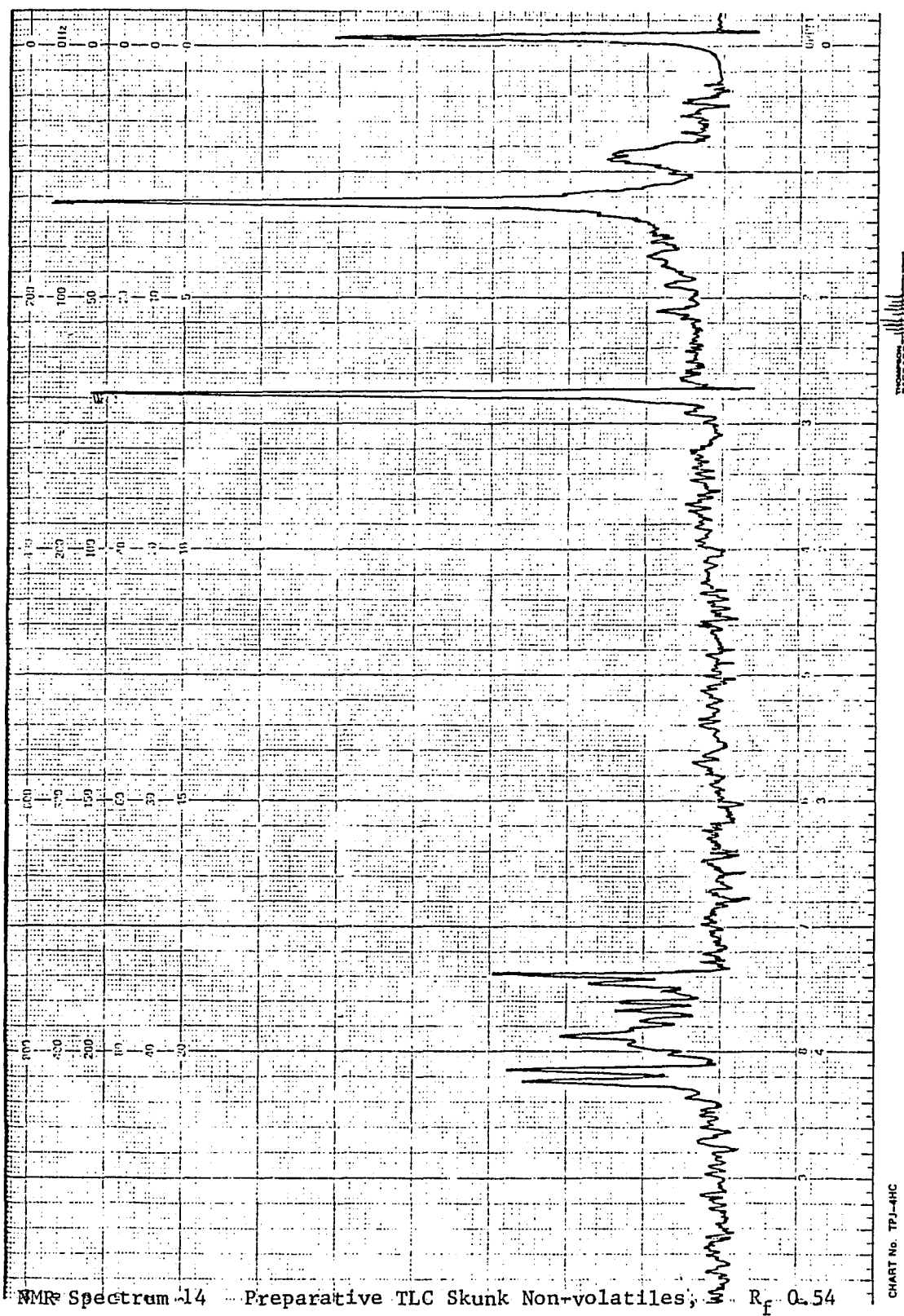


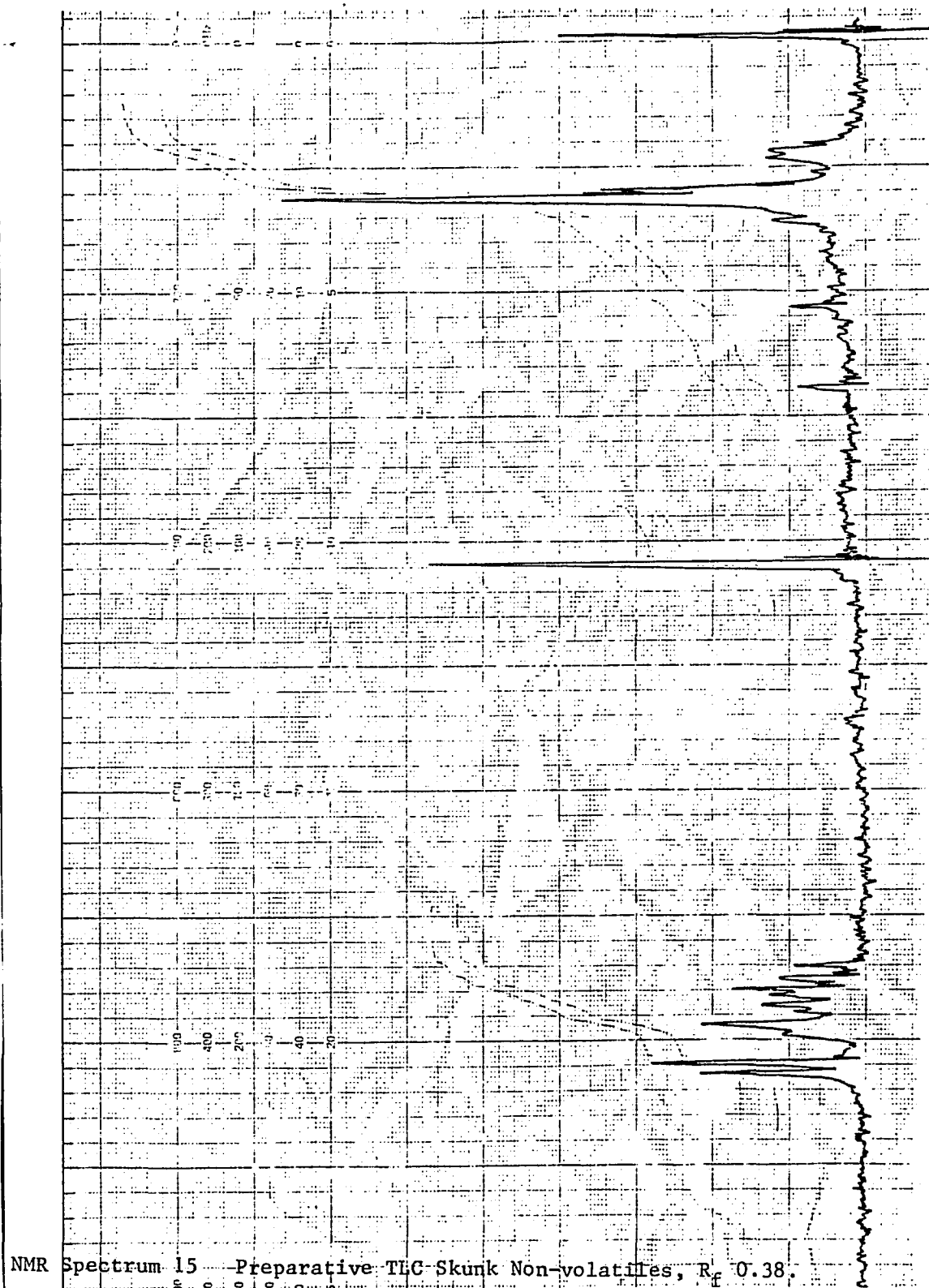
NMR Spectrum 12

Column Chromatogram of Skunk Non-volatiles,
Fraction 27.

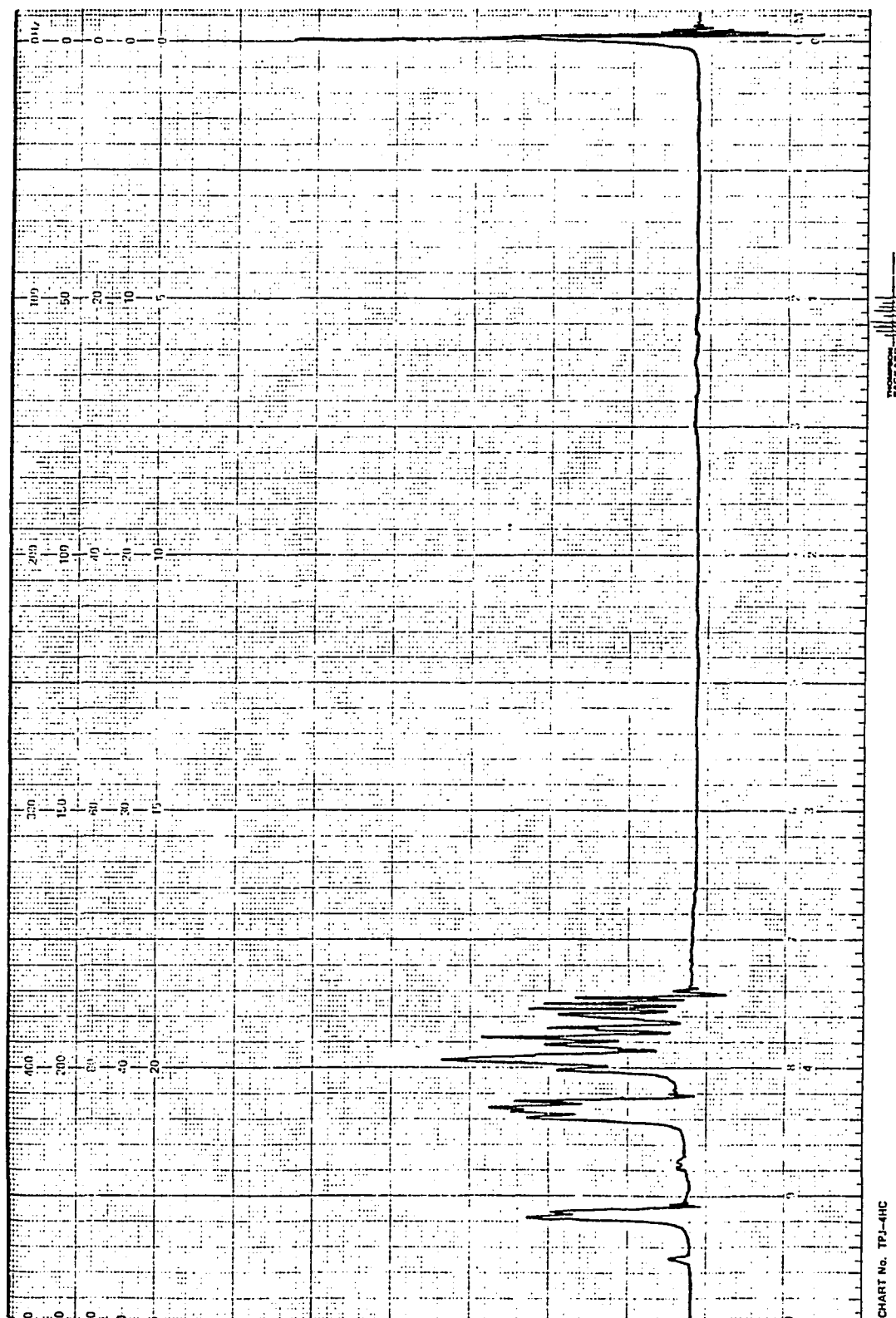


NMR Spectrum 13 Column Chromatogram of Skunk Non-volatiles,
Fraction 30.

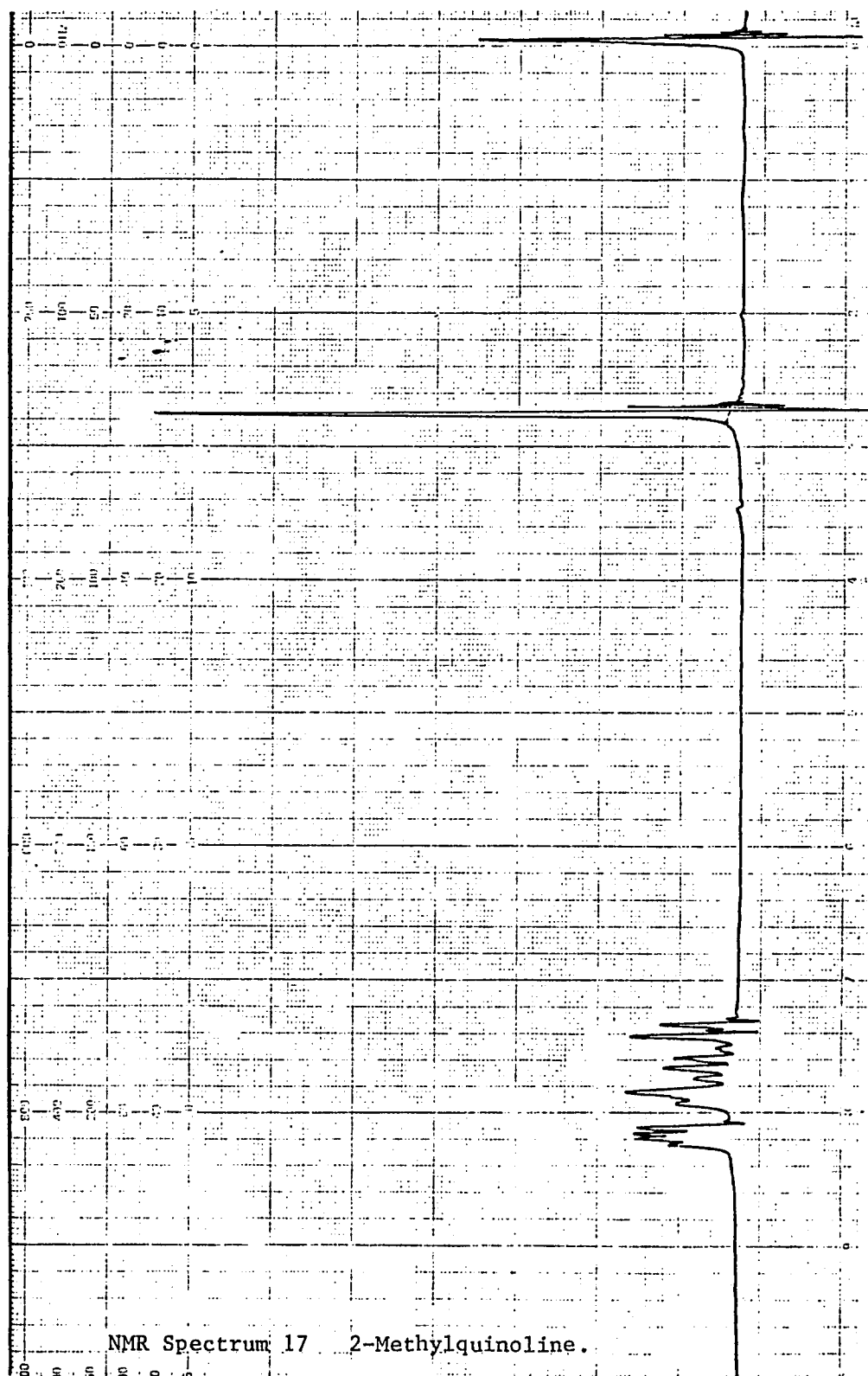


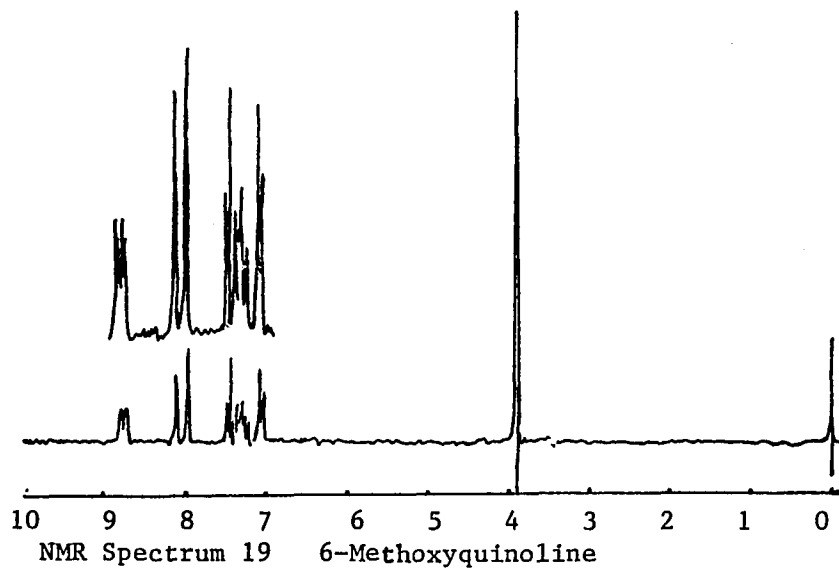
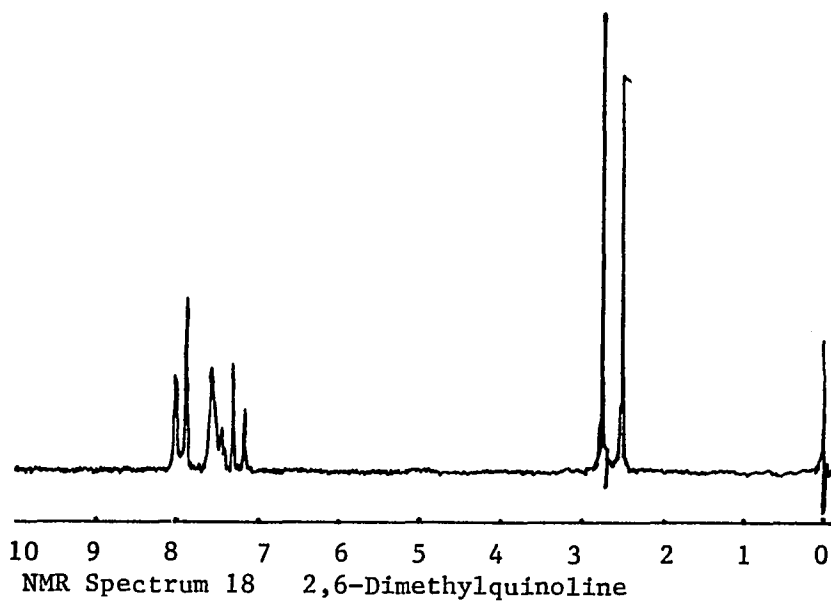


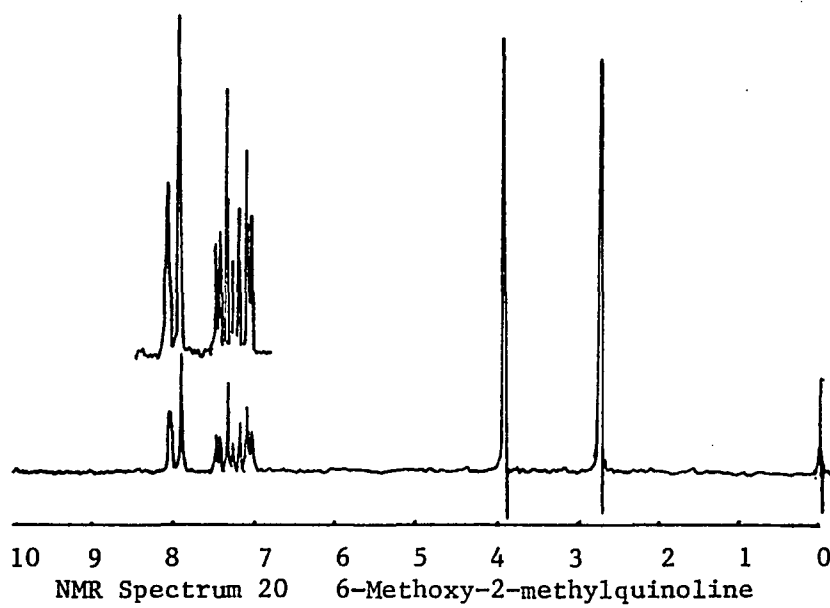
NMR Spectrum 15 — Preparative TLC Skunk Non-volatiles, R_f 0.38.



NMR Spectrum 16 Quinoline.







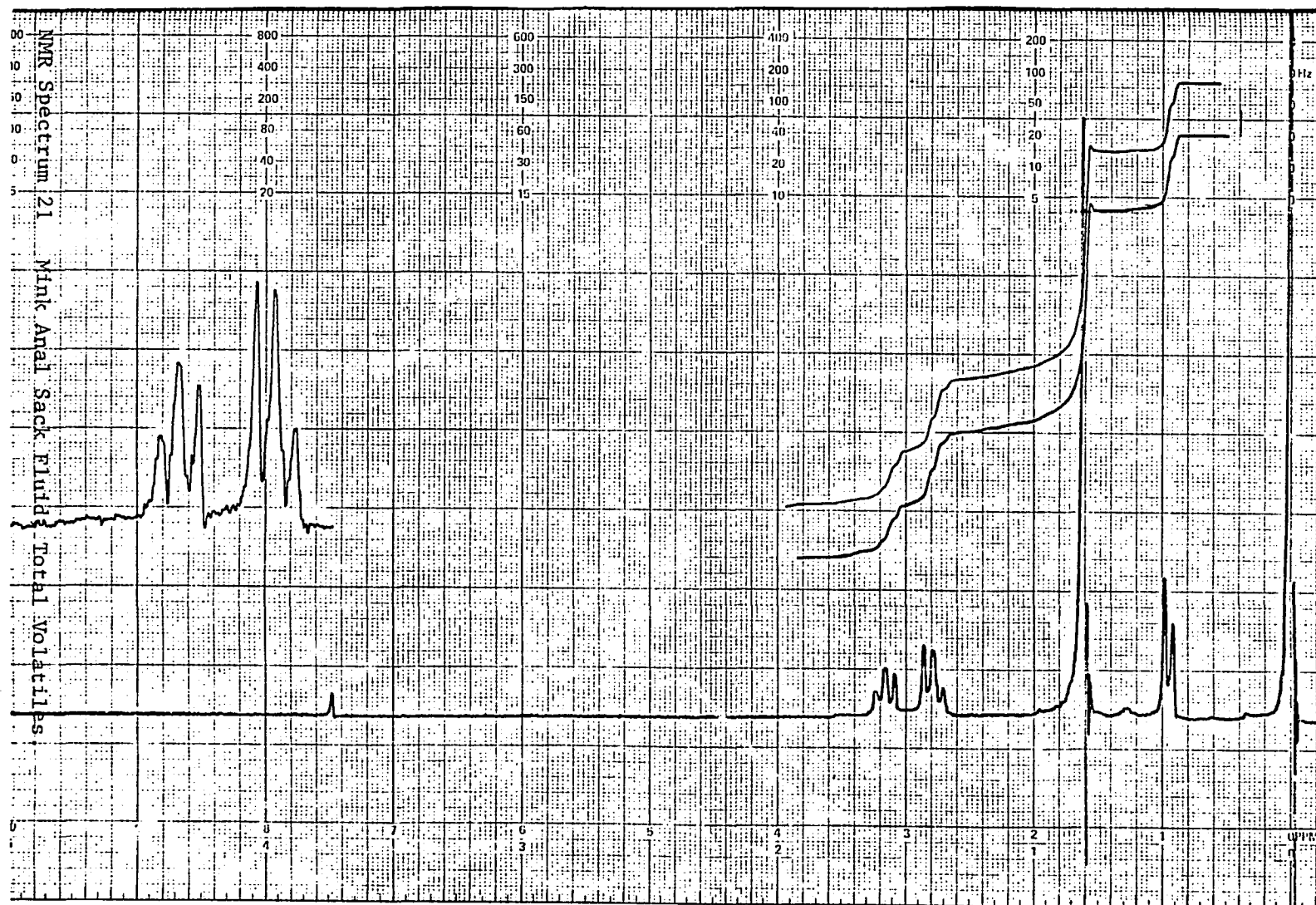
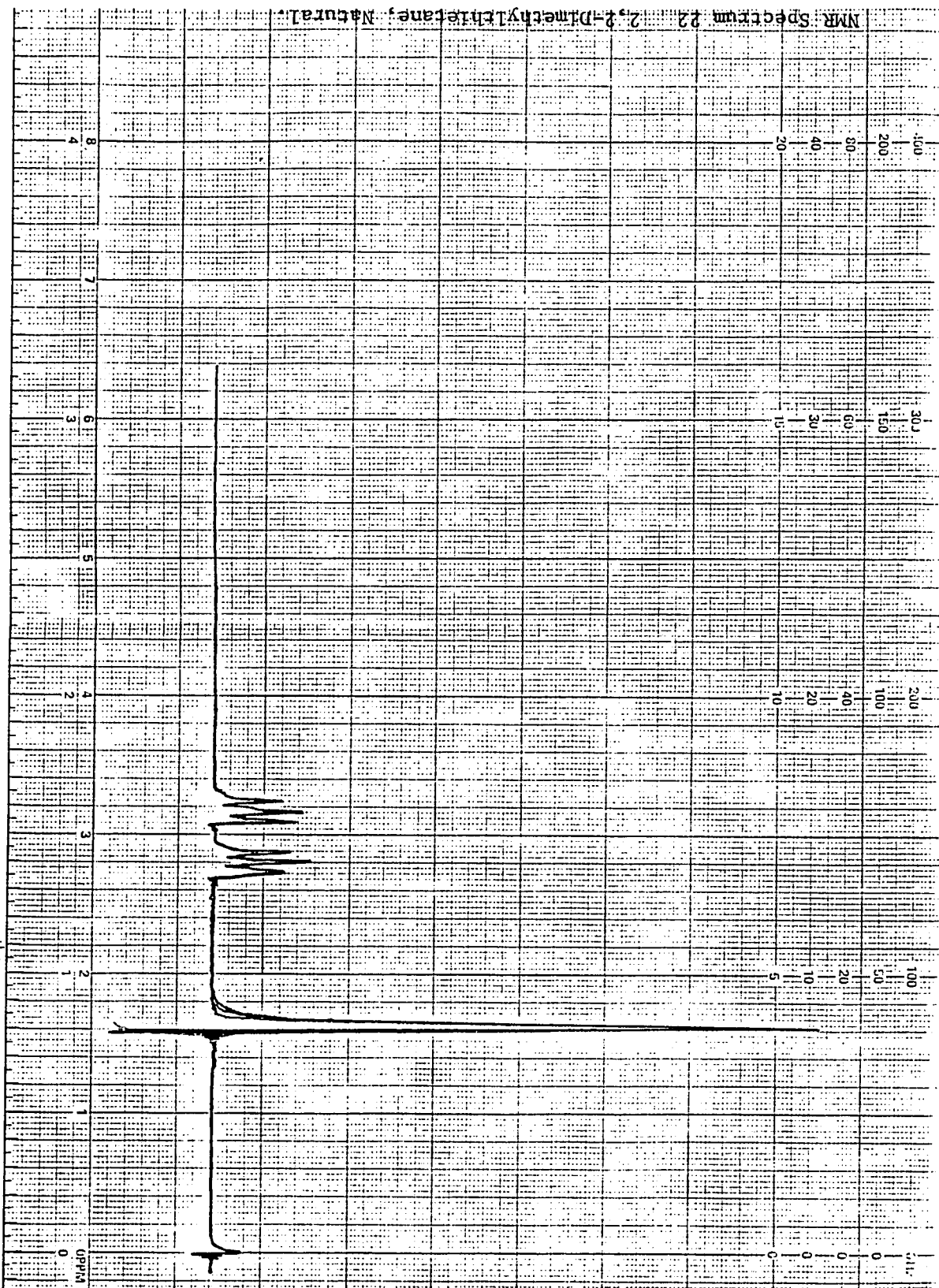


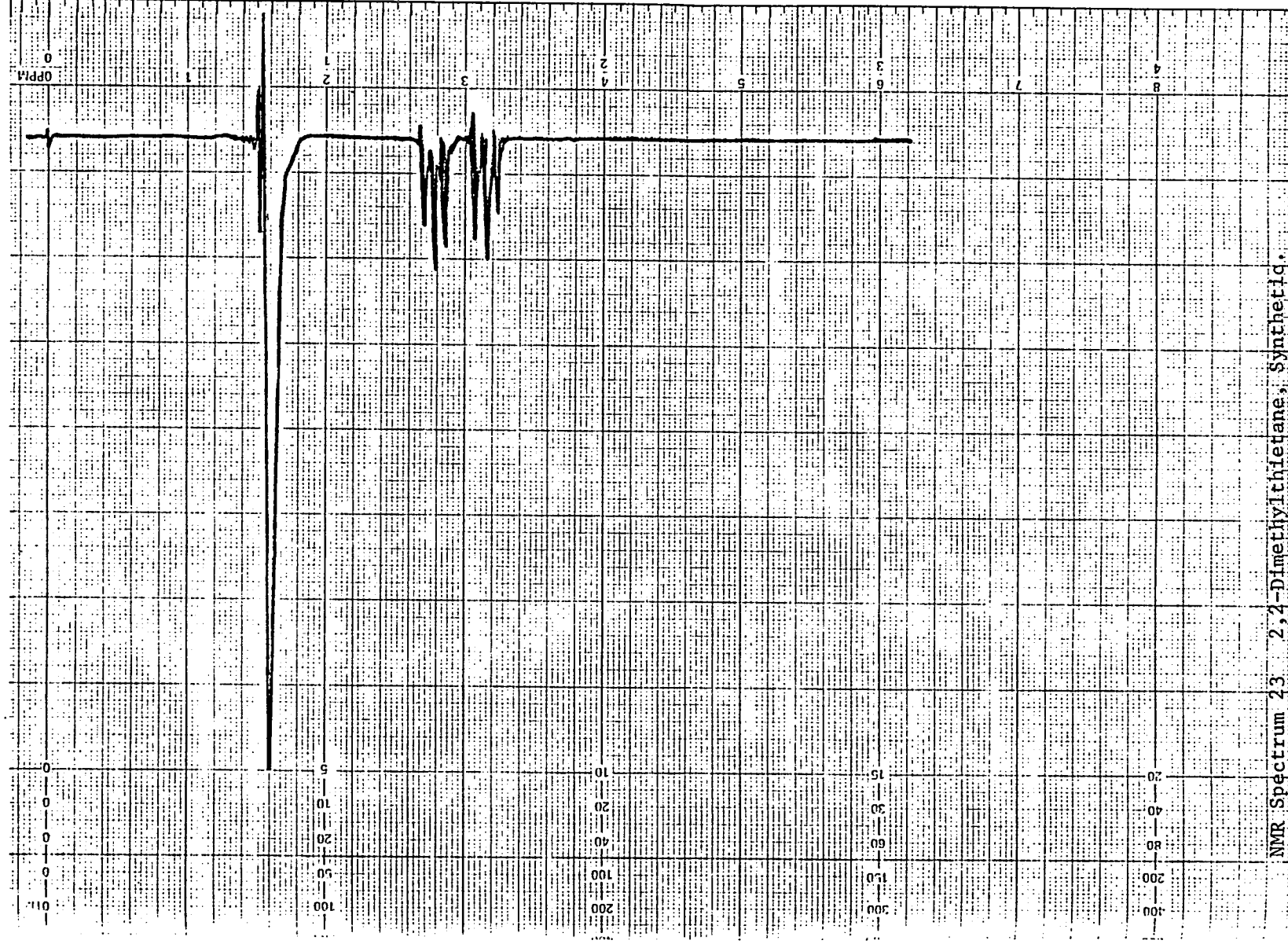
CHART No. TPJ-4HC

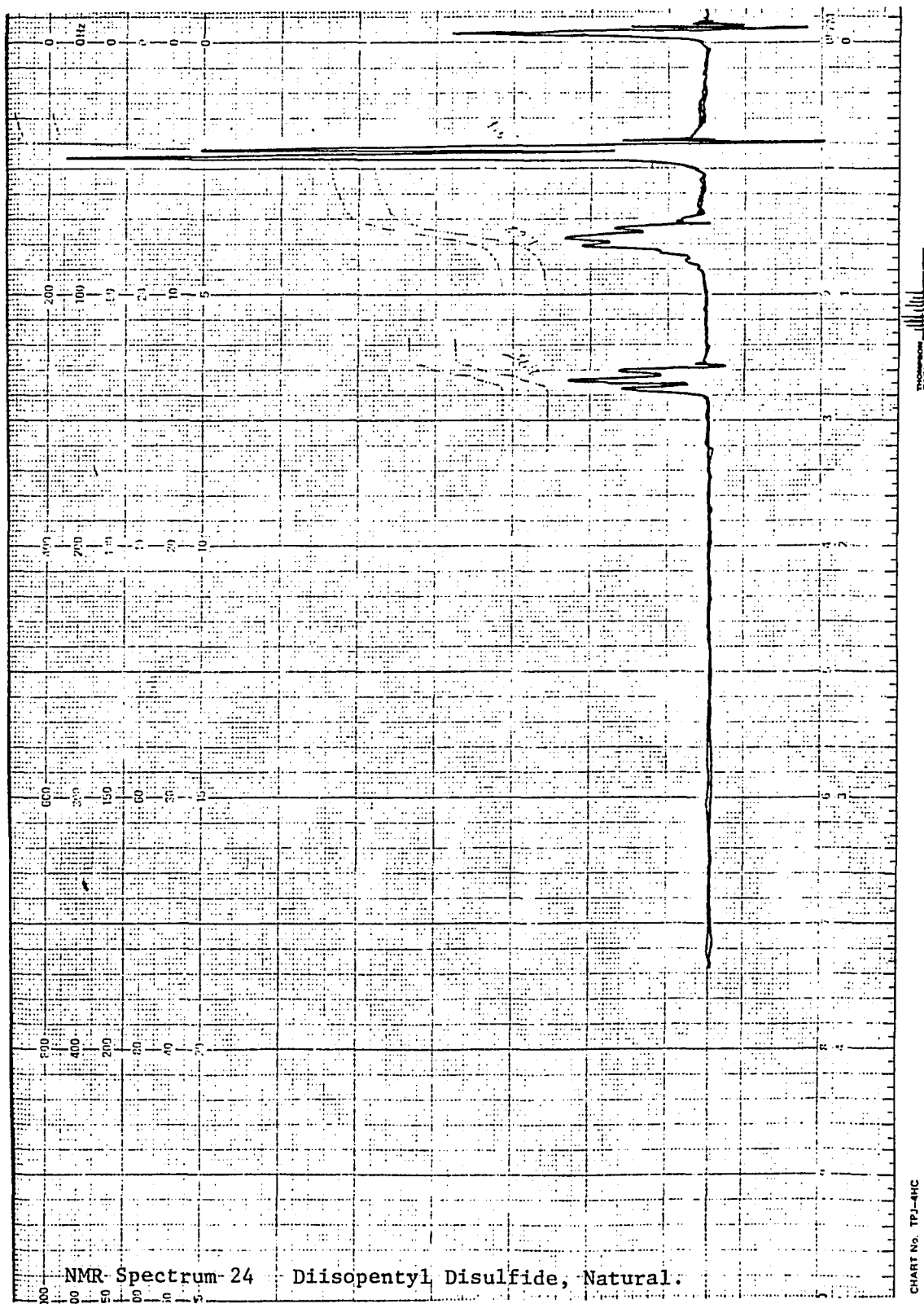
THOMPSON
PACKARD

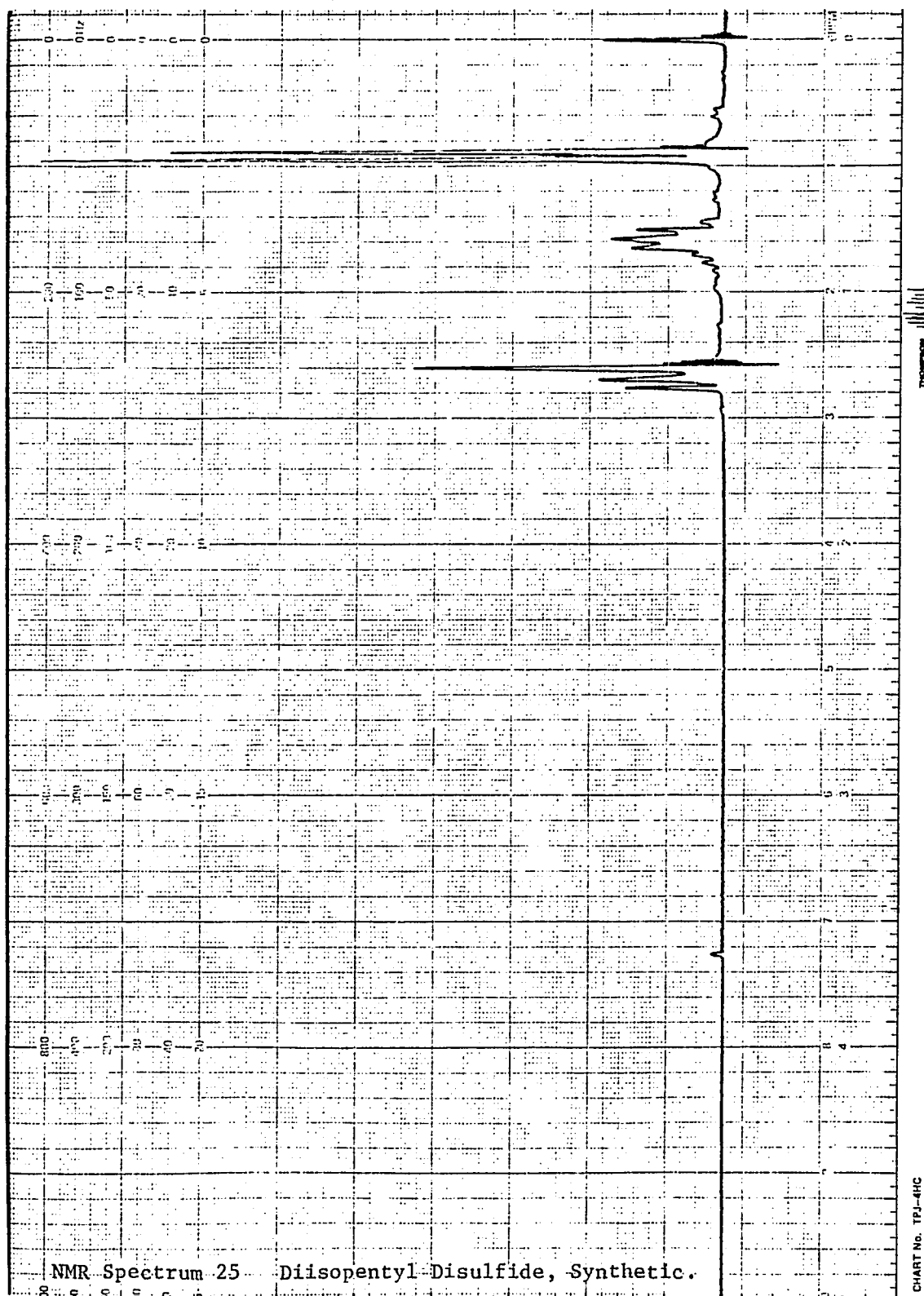
NMR Spectrum 22 2,2-Dimethylthietane, Natural.

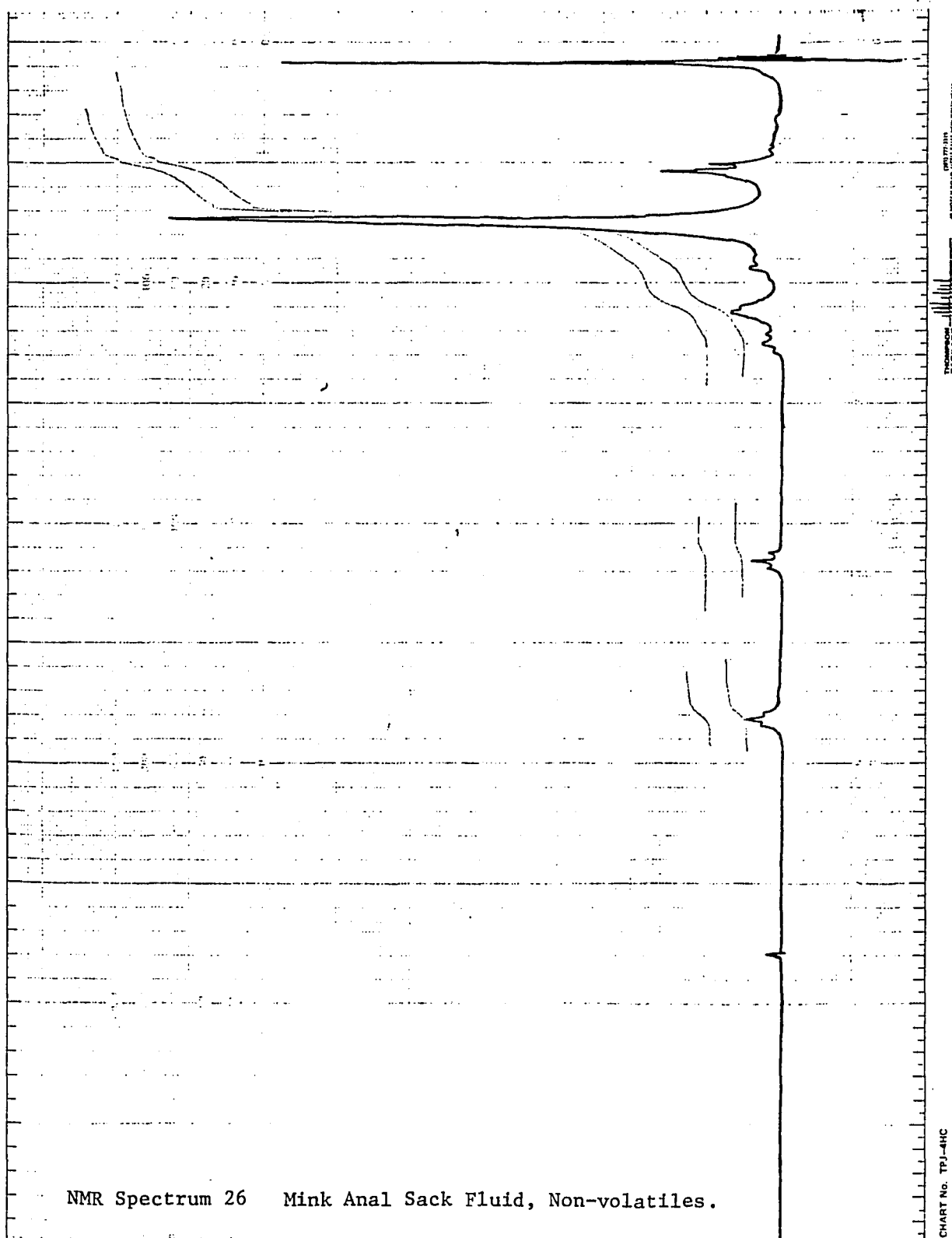


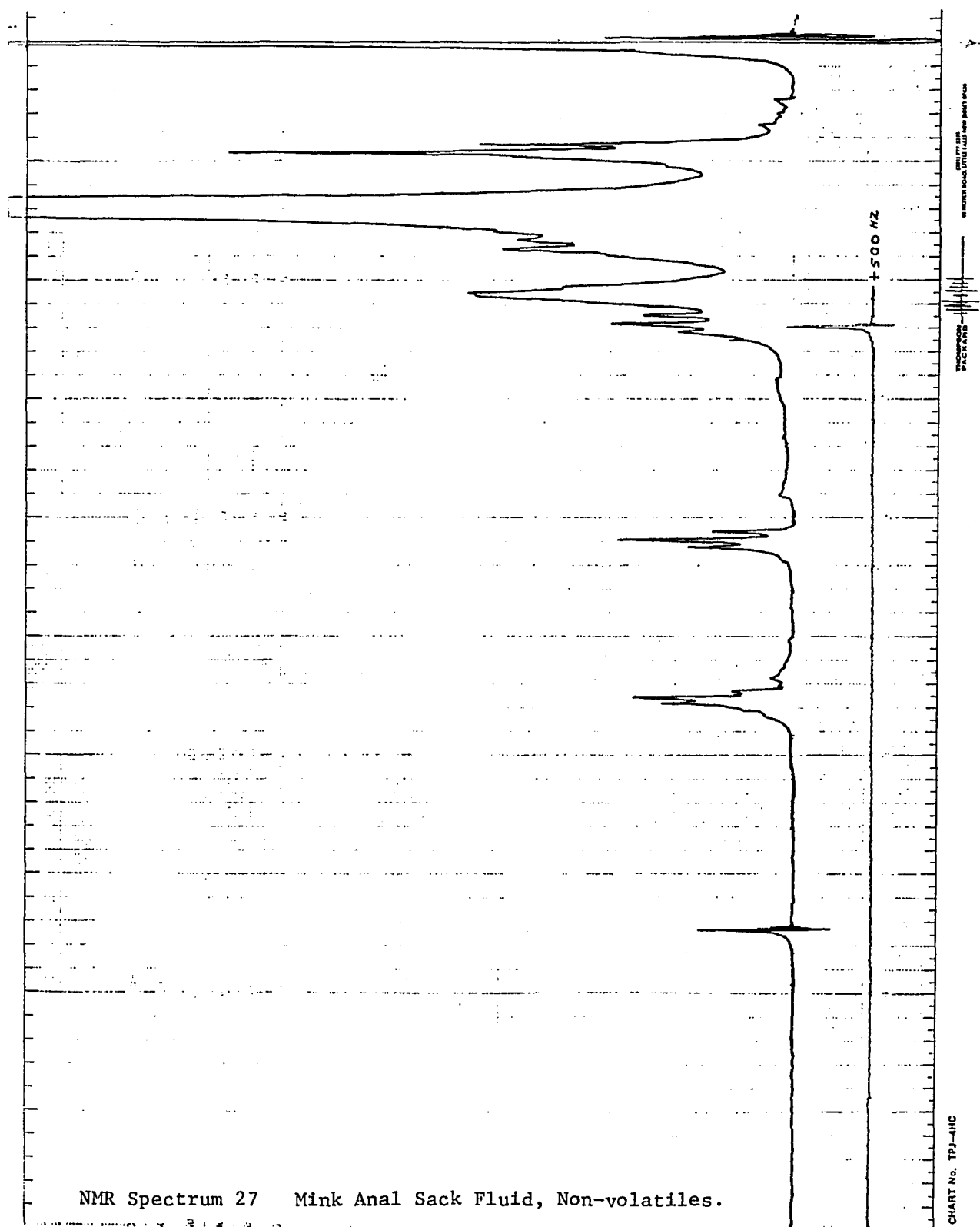
TECHNICAL
PACK AND
40 MICRONS SOLID STATE NMR SPECTROMETER

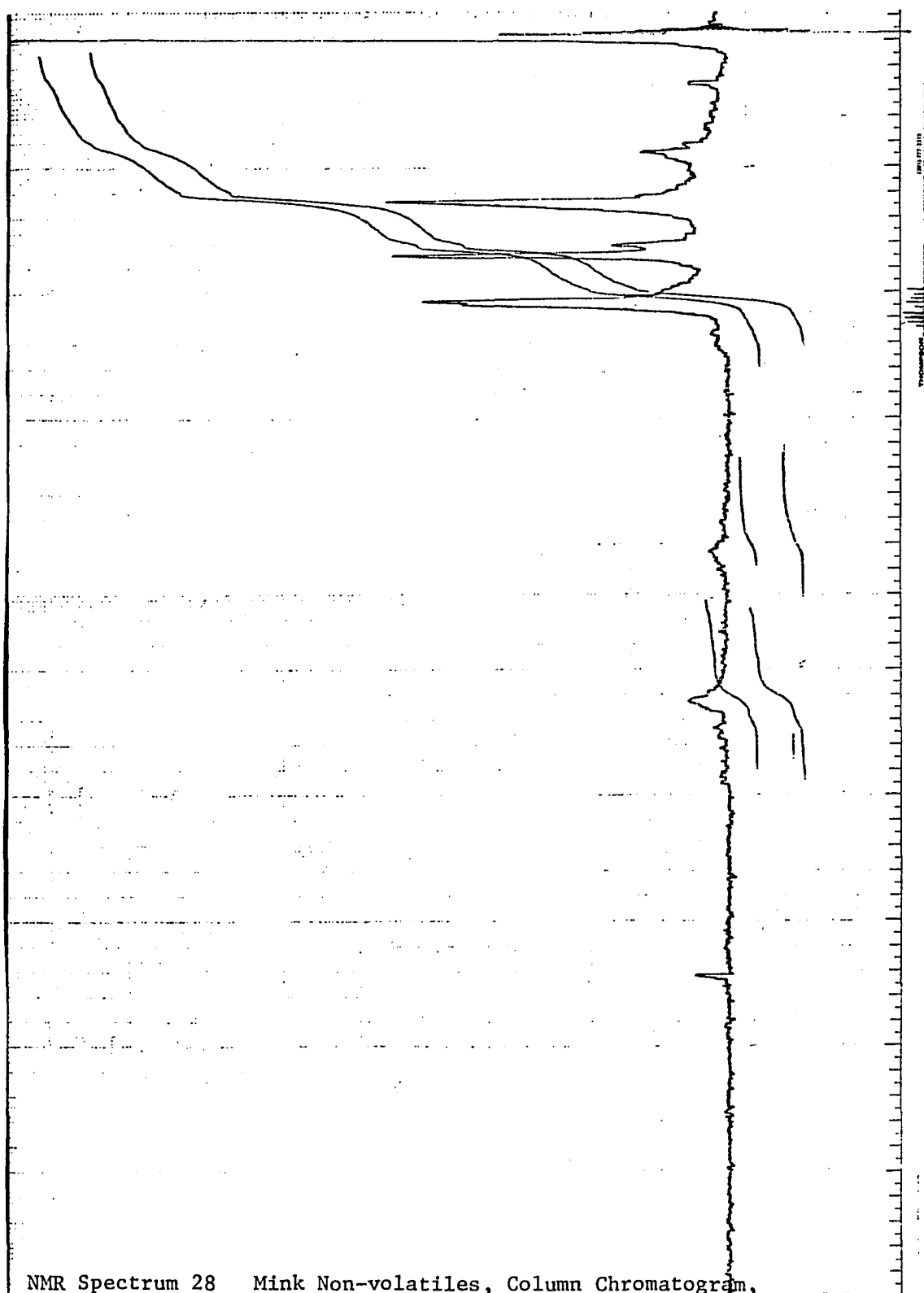


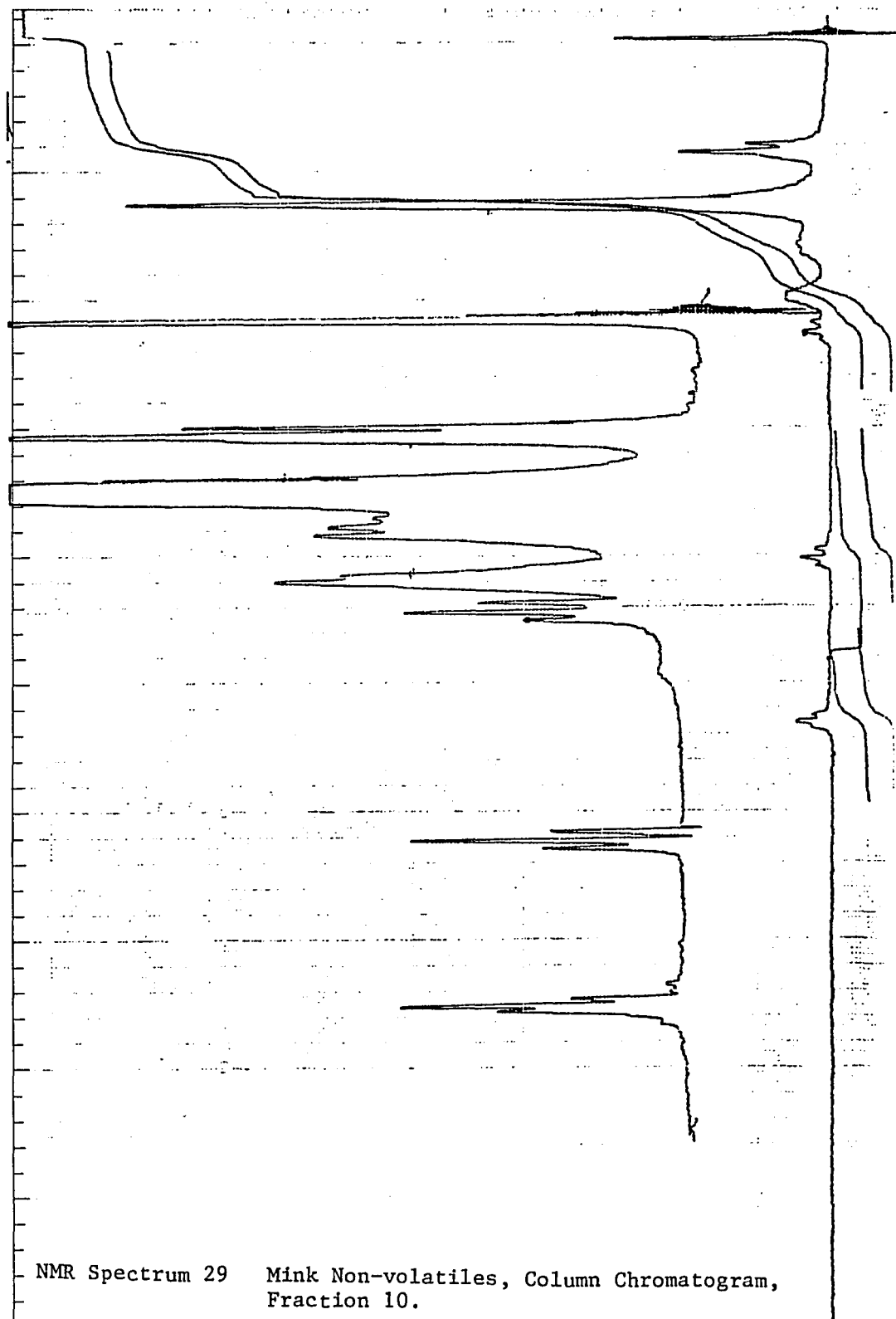


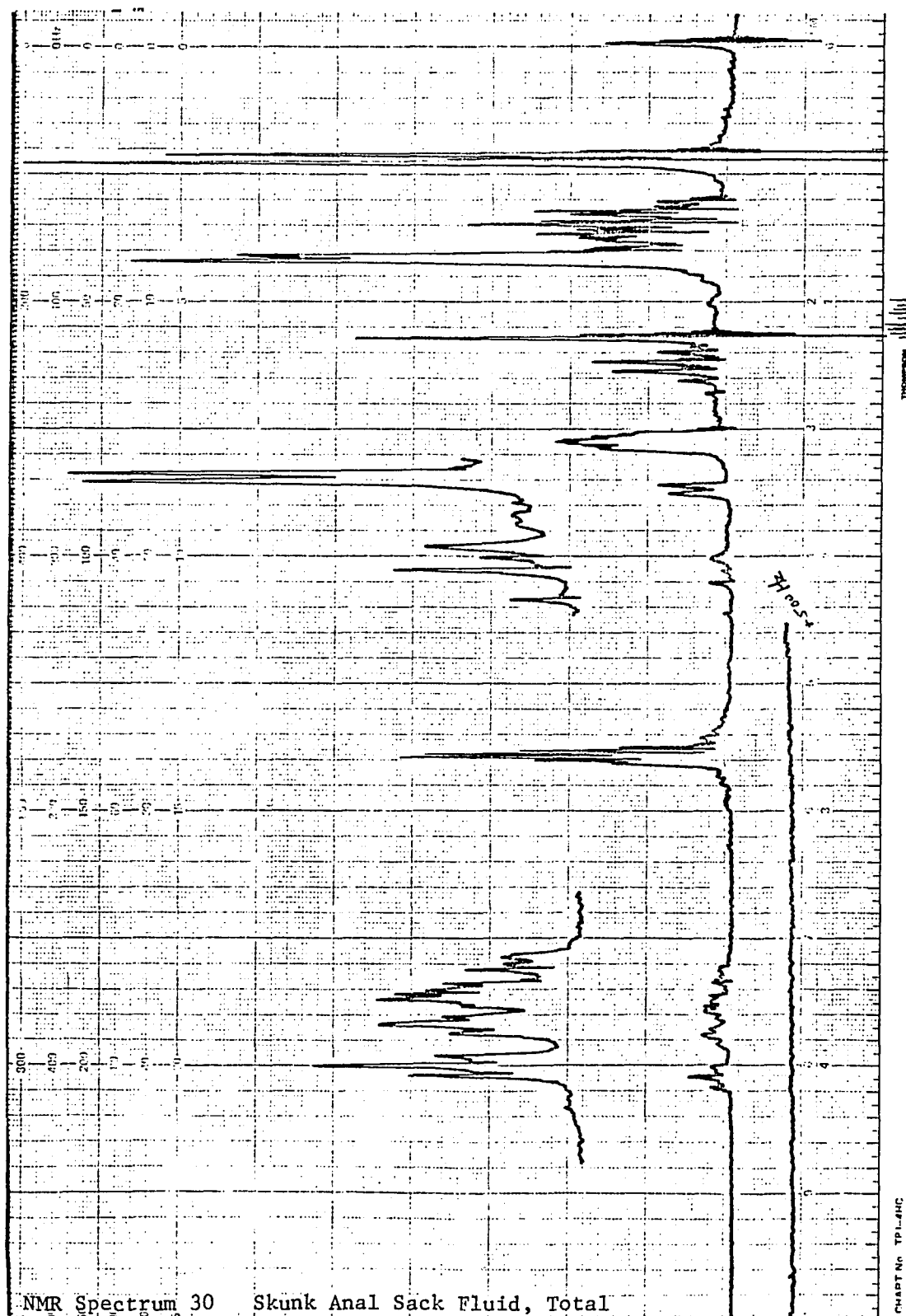


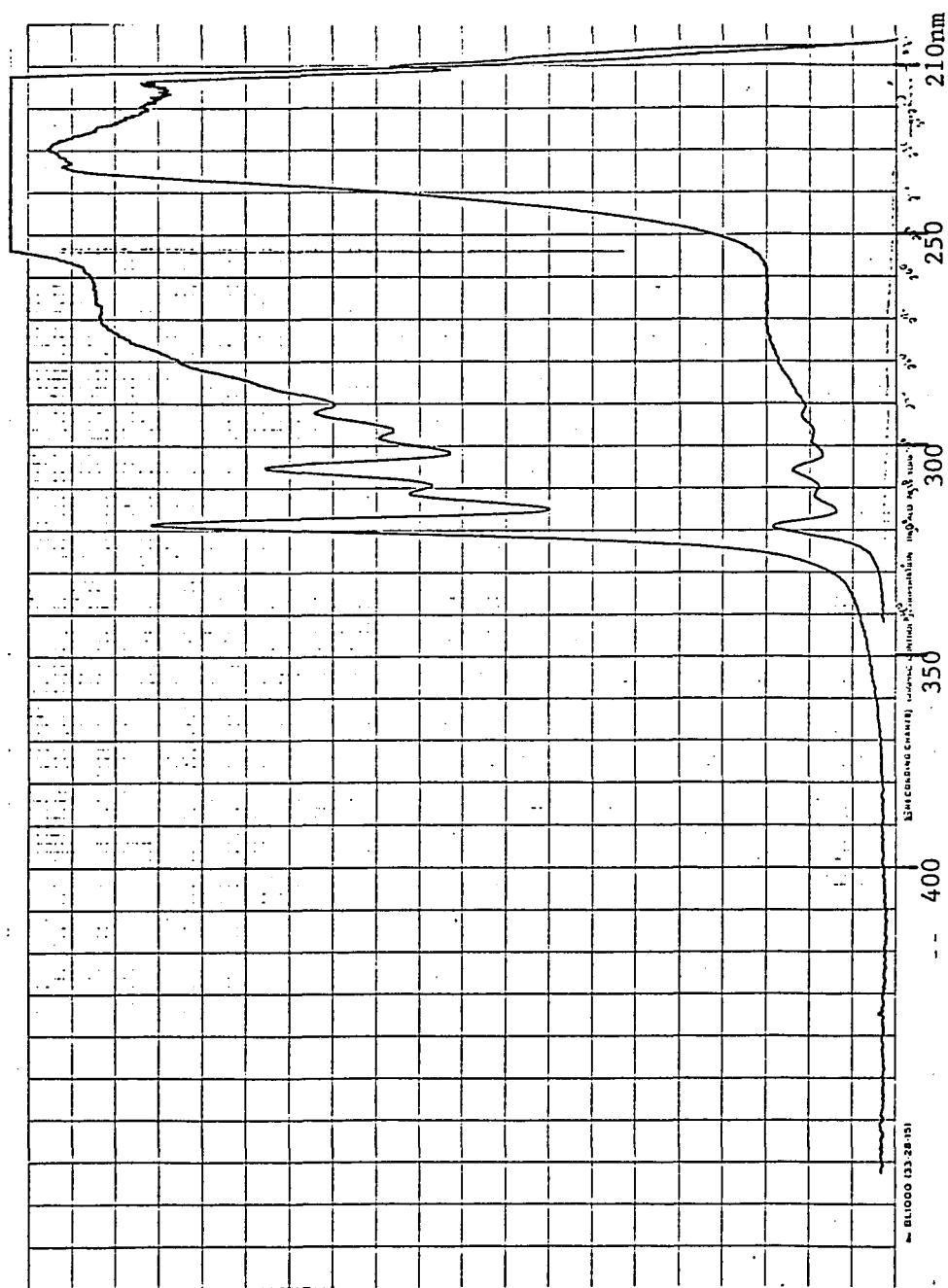




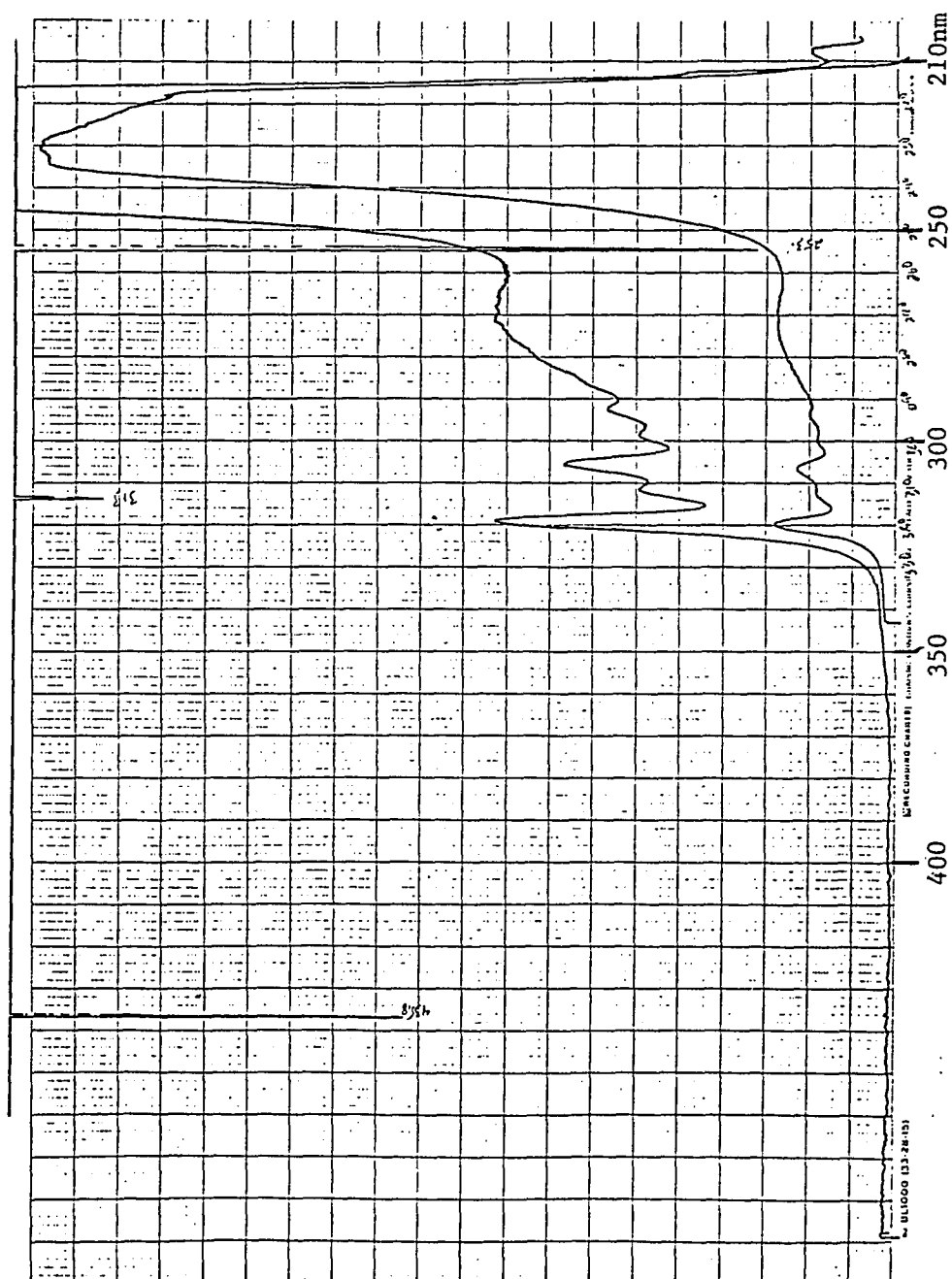




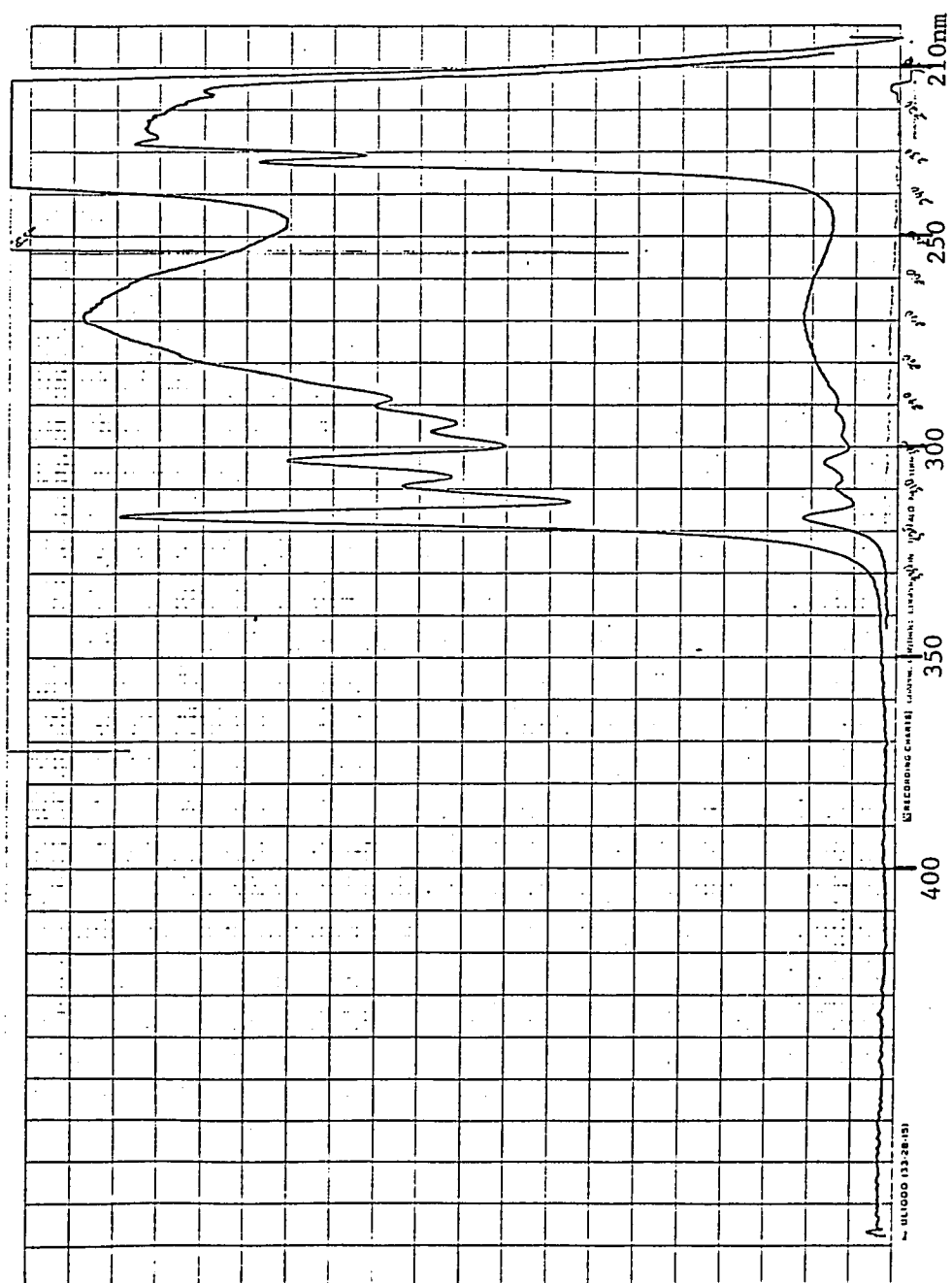




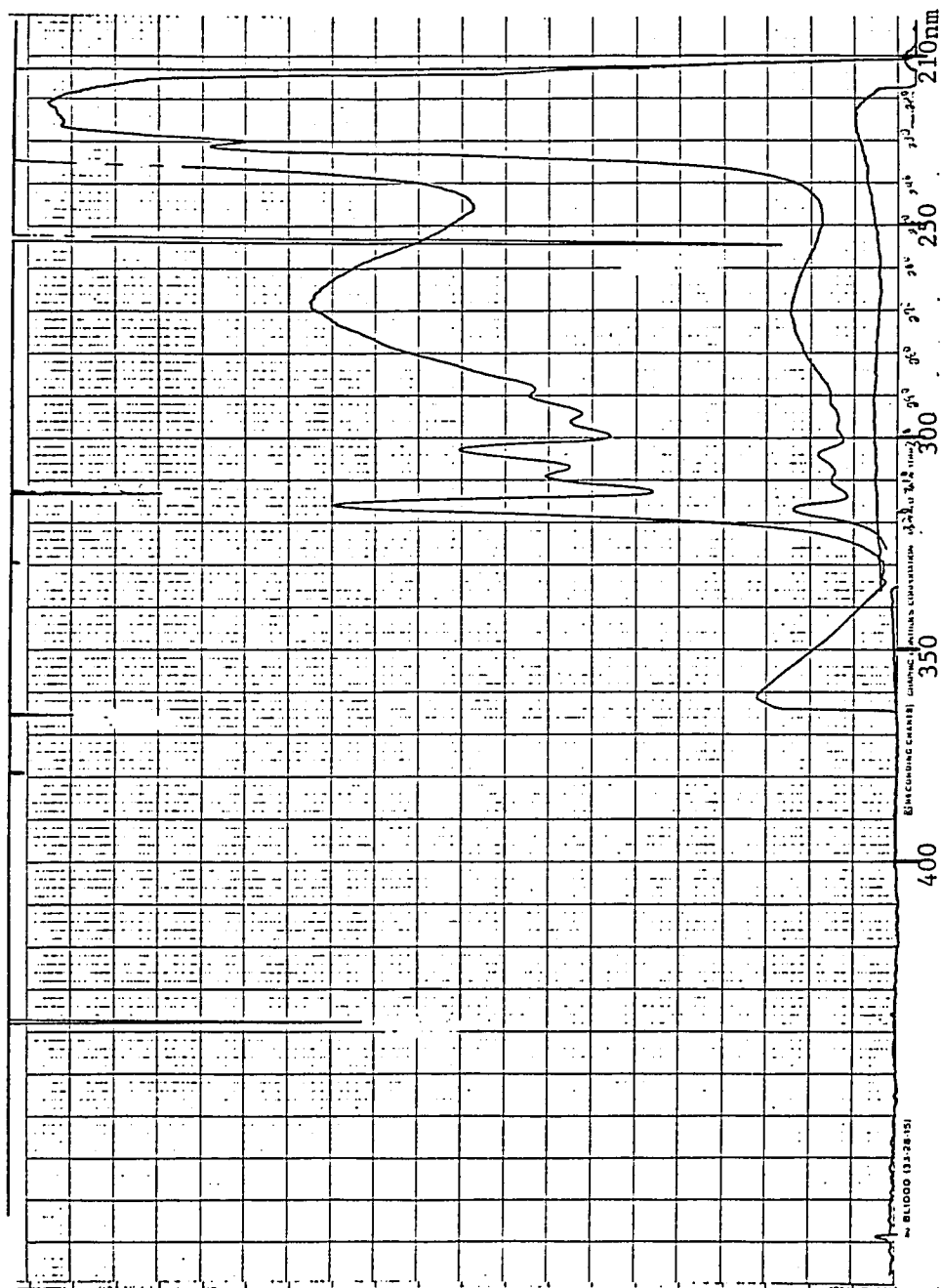
UV Spectrum 1 Preparative TLC, R_f 0.54 (cyclohexane).



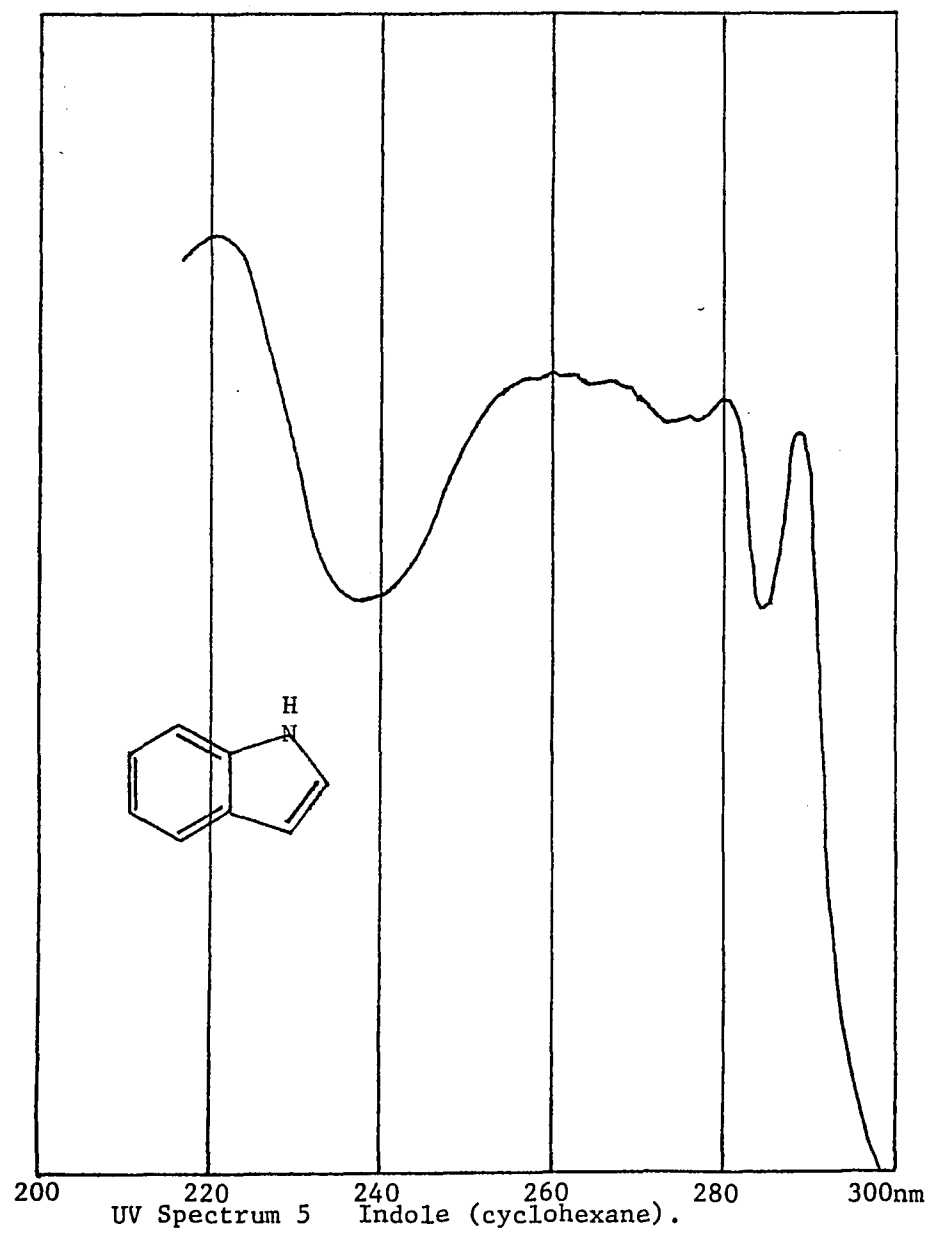
UV Spectrum 2 Preparative TLC, R_f 0.54 (ether).

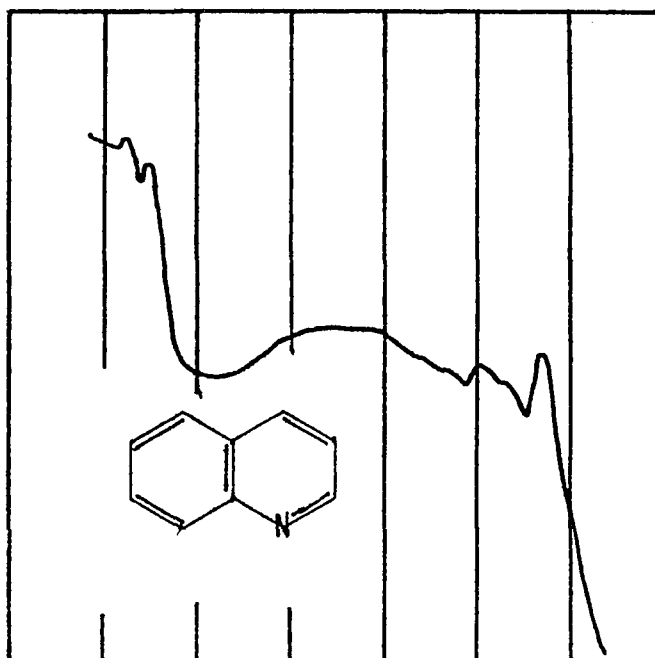


UV Spectrum 3 Preparative TLC, R_f 0.38 (cyclohexane).

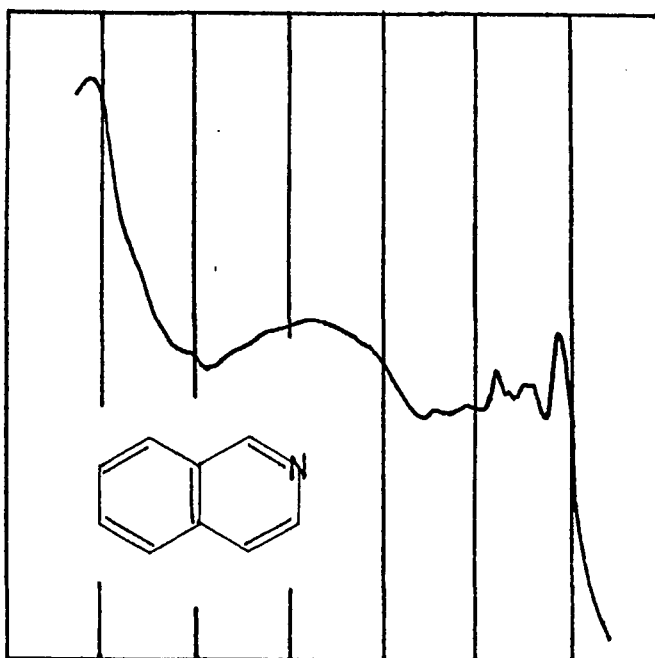


UV Spectrum 4 Preparative TLC, R_f 0.38 (ether).

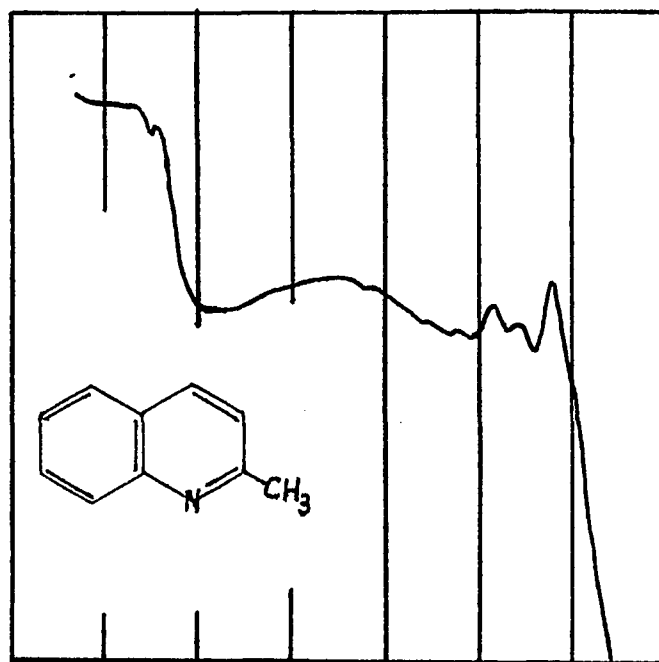




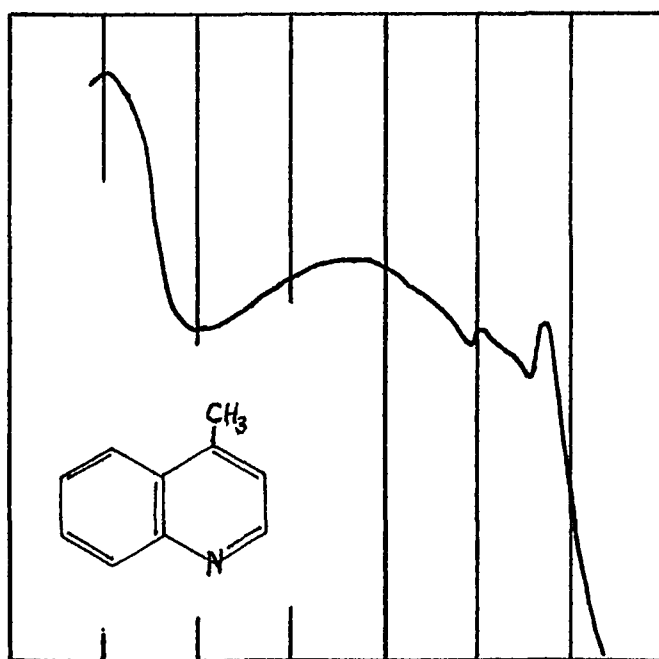
200 220 240 260 280 300 320 340(nm)
UV Spectrum 7 Quinoline (cyclohexane)



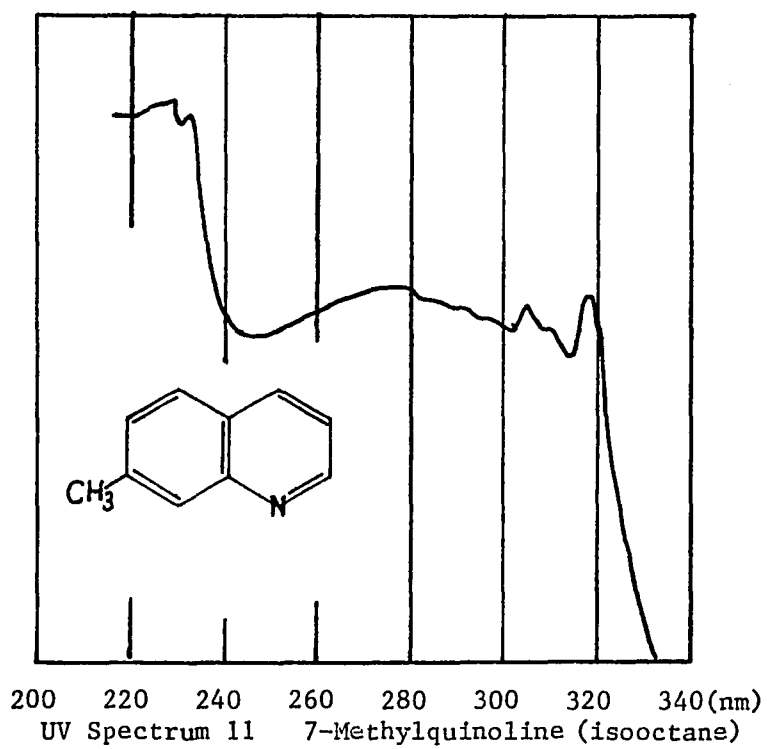
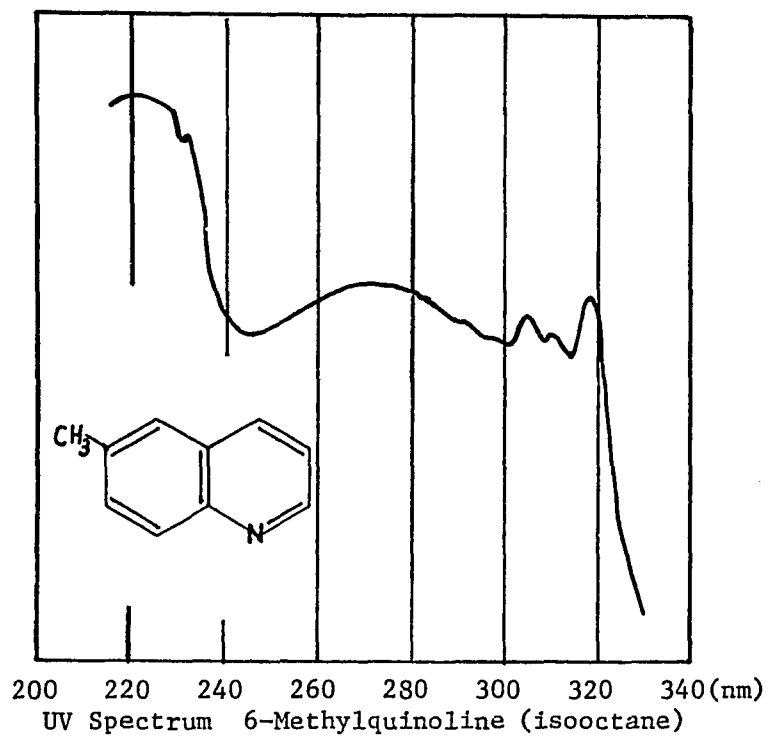
200 220 240 260 280 300 320 340(nm)
UV Spectrum 6 Isoquinoline (cyclohexane)

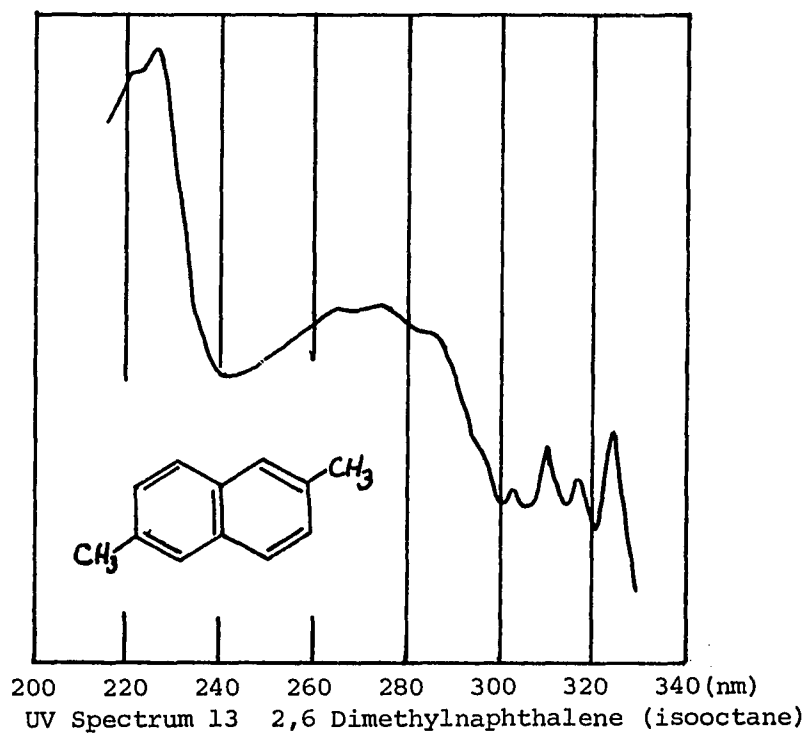
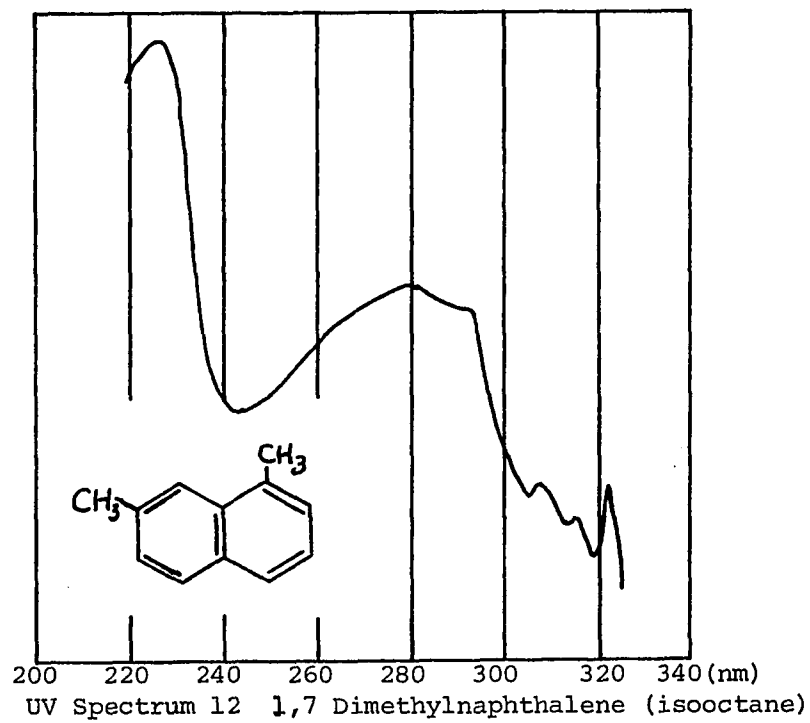


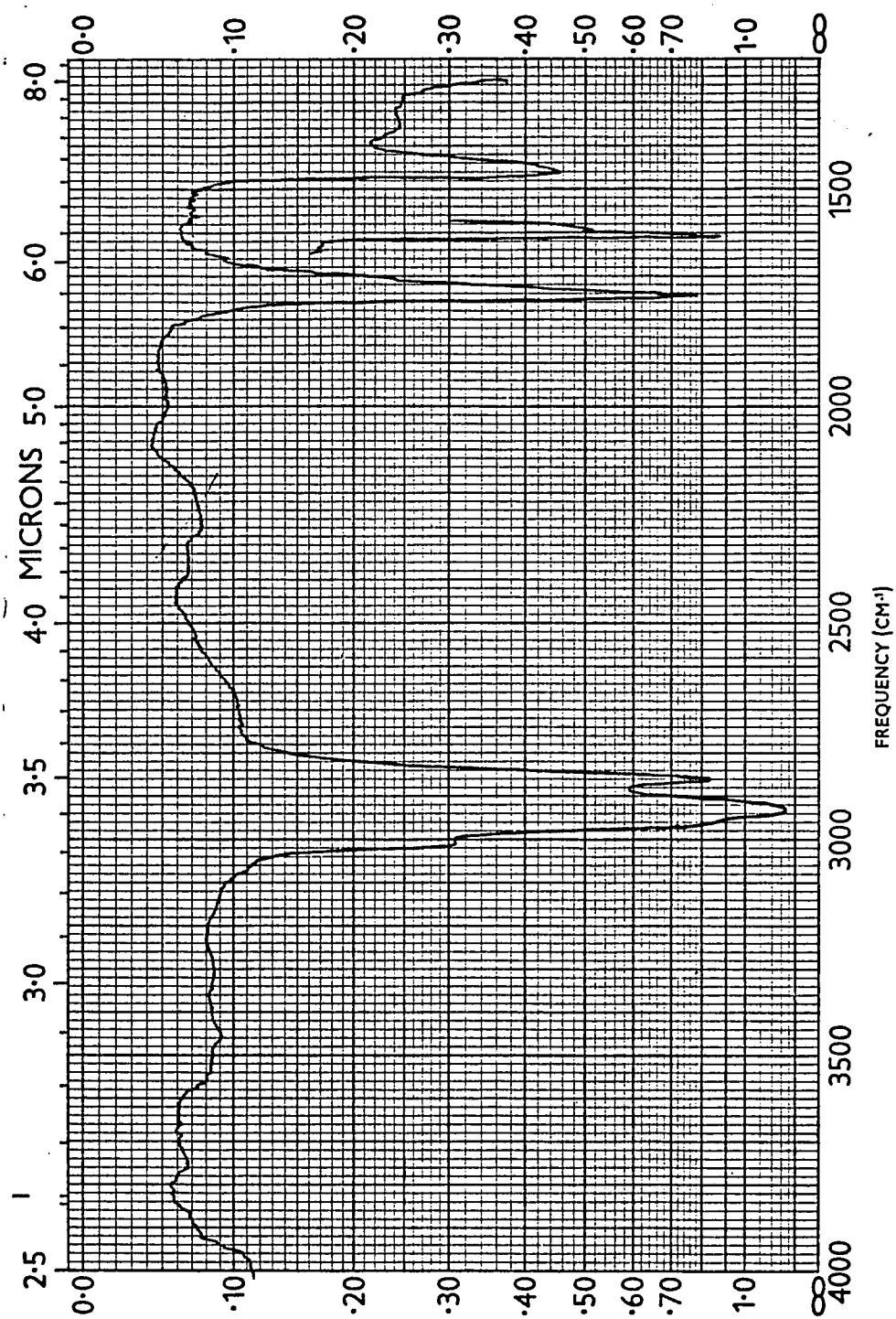
200 220 240 260 280 300 320 340(nm)
UV Spectrum 8 2-Methylquinoline (isooctane)



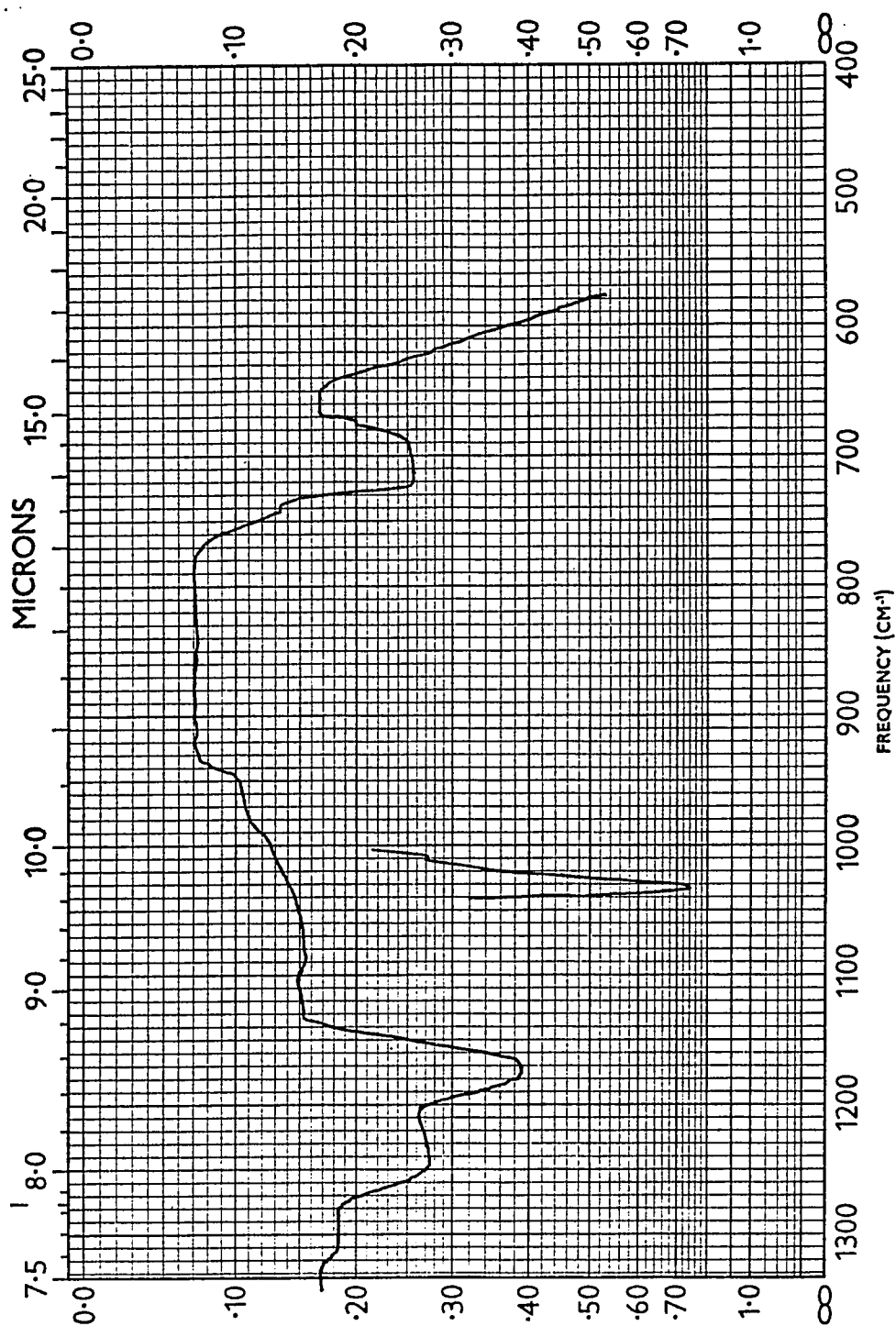
200 220 240 260 280 300 320 340(nm)
UV Spectrum 9 4-Methylquinoline (isooctane)



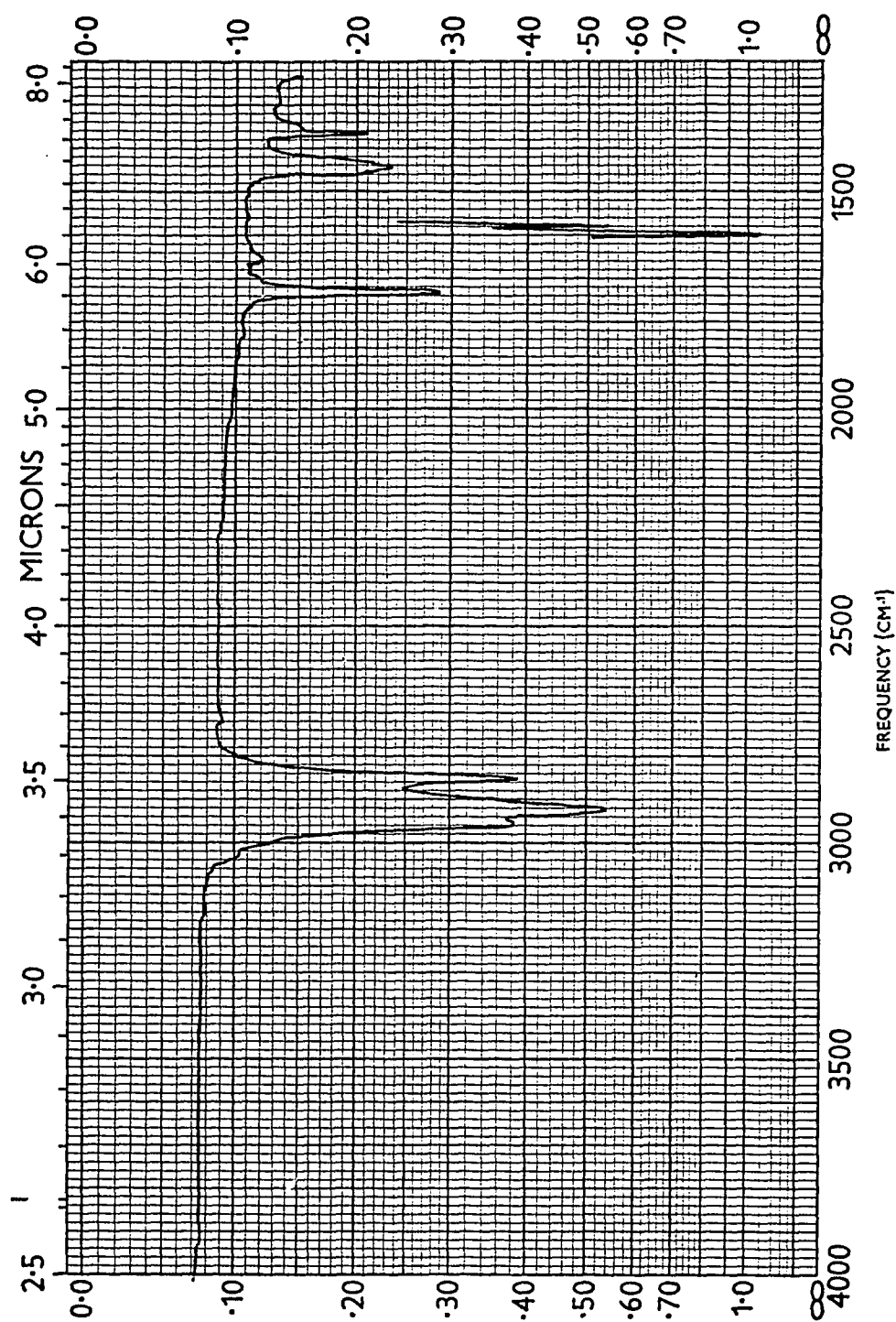




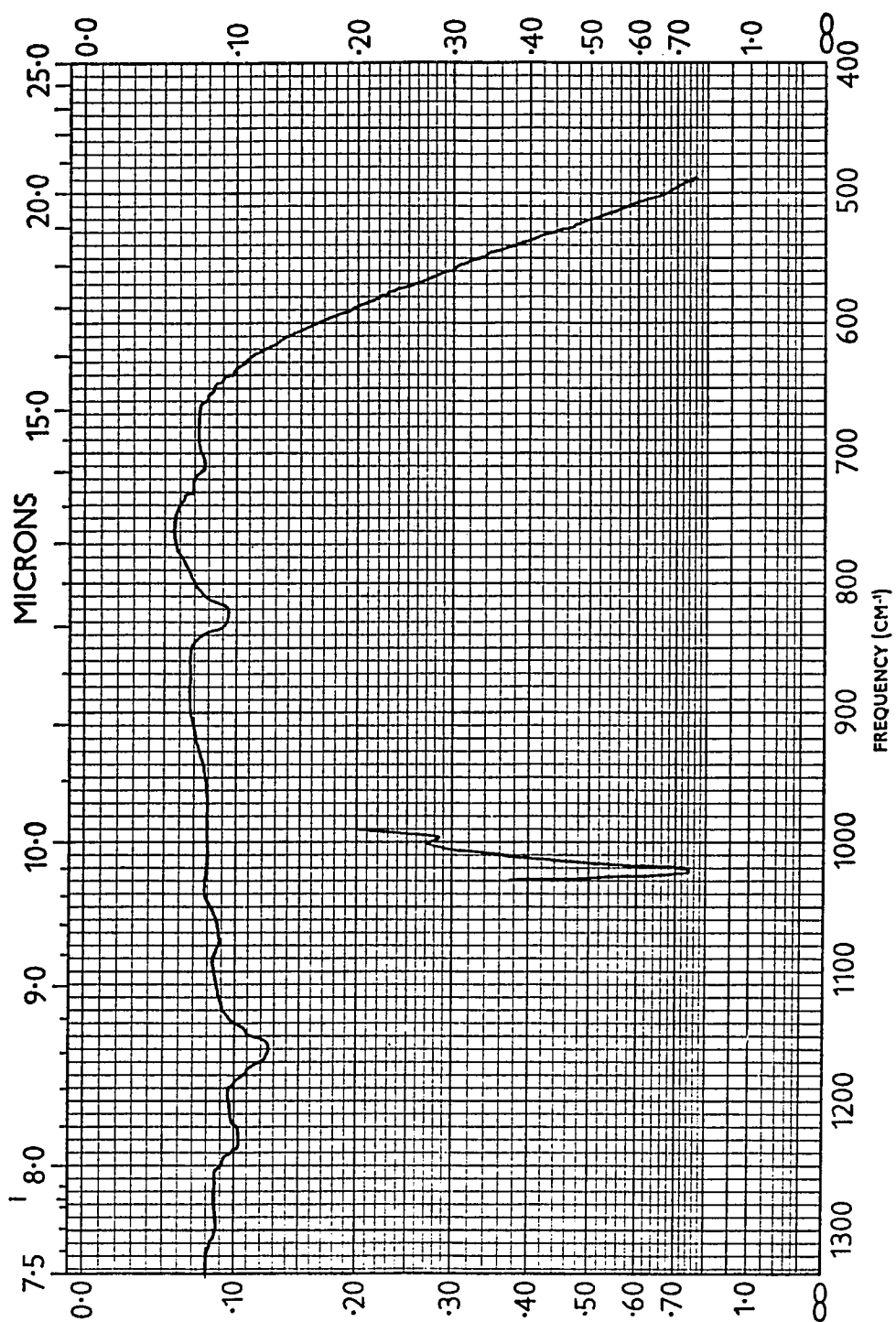
Infrared Spectrum 1 Mink Nonvolatiles (total).



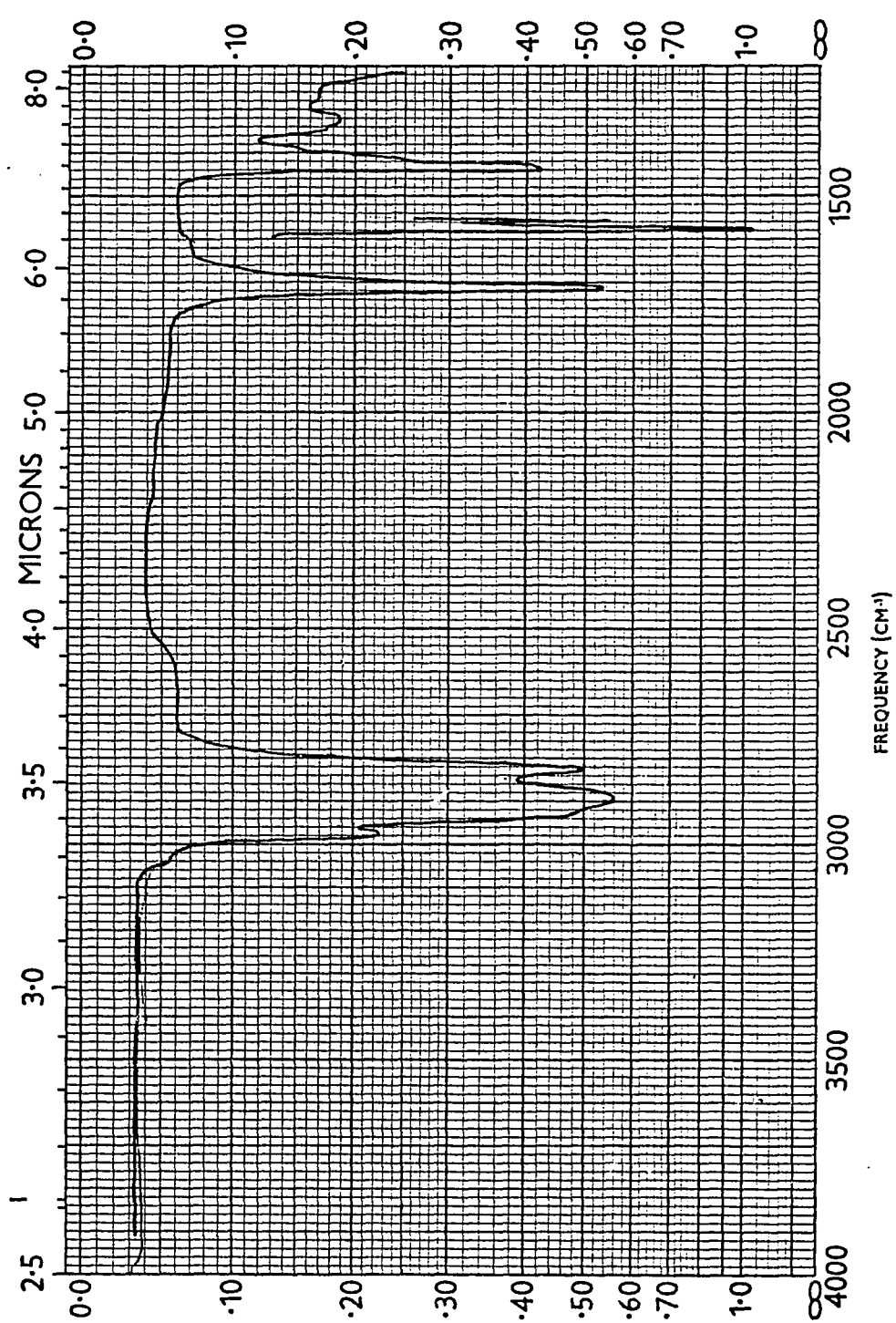
Infrared Spectrum 1 Mink Nonvolatiles (total).



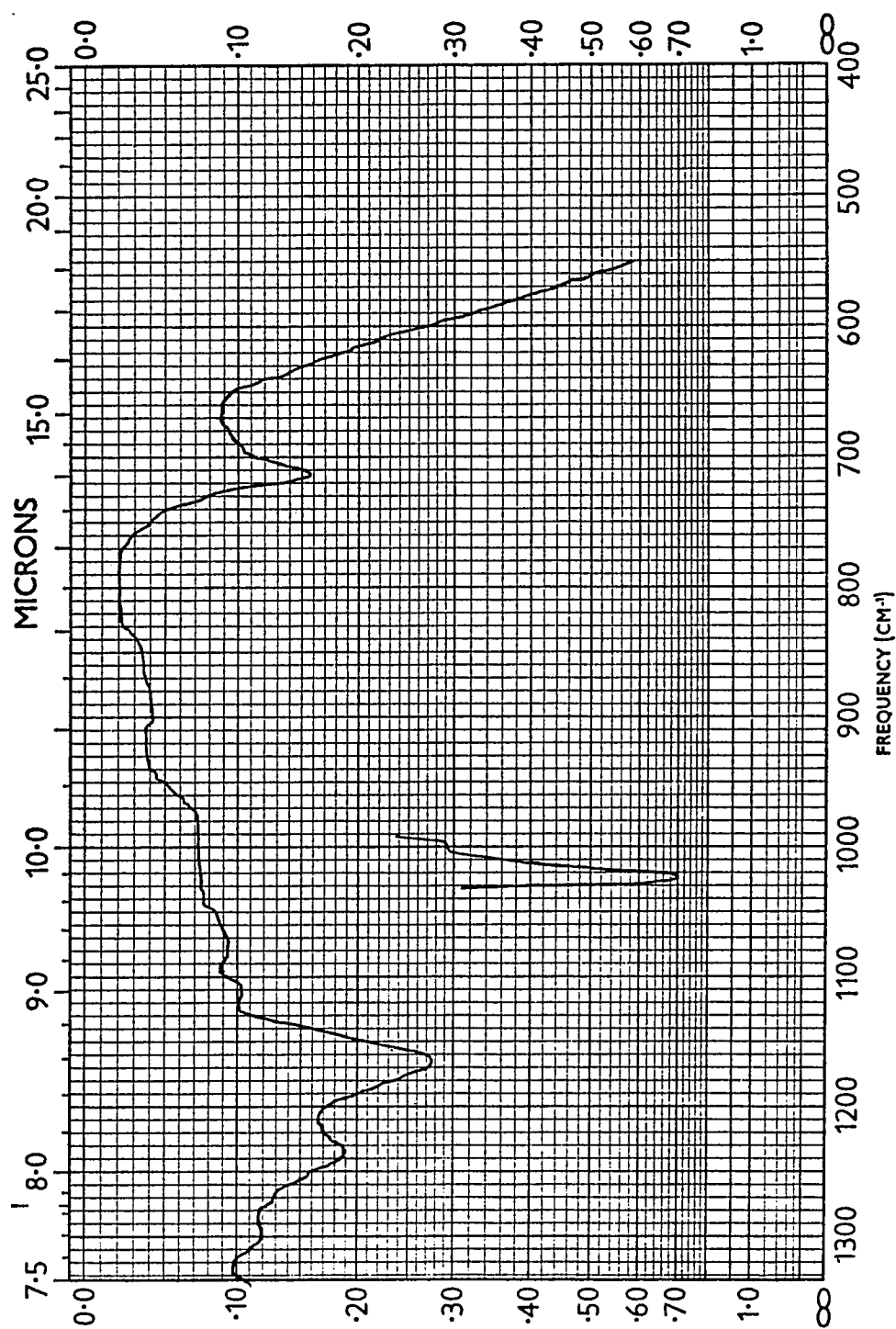
Infrared Spectrum 2 Mink Nonvolatiles (fraction 7).



Infrared Spectrum 2 Mink Nonvolatiles (fraction 7).



Infrared Spectrum 3 Mink Nonvolatiles (fraction 10).



Infrared Spectrum 3 Mink Nonvolatiles (fraction 10).